CONFERENCE PROGRAM

09.10.2020 Friday

09:00-09:10 Opening ceremony – Prof. Jolanta Saczko and Prof. Agnieszka Piwowar (Vice-Rector for Educational Affairs)
available all the time VIRTUAL POSTER SESSION

1st SESSION

Moderators: Jolanta Saczko and Agnieszka Piwowar

09:10-09:55 Lecture of Prof. Mounir Tarek (CNRS – University de Lorraine, Nancy, France) *Frontiers of Electroporation, from Mechanisms to Applications: Unraveling new key molecular level aspects using computational chemistry*

10:00-11:00 Presentation of young scientists

S1.O1. *Relationship between the concentration of IL-6, insulin activity, glycated haemoglobin in human blood and the development of type 2 diabetes and/or obesity*, Magdalena Król
S1.O2. *Assessment of the stimulation level of the antitumor response by dendritic cells modified to overexpression of IL-12 and/or IL-18 in vitro*, Węgierek Katarzyna
S1.O3. *Development of new polymeric materials with the incorporated API for potential application in solid dosage forms formulation using 3D printing technology*, Kozakiewicz Marta
S1.O4. *Antitumor activity of therapy composed of methotrexate nanoconjugate and dendritic cell-based vaccines and its influence on local and systemic antitumor immune response*, Szczygieł Agnieszka
S1.O5. *Regulation of mitochondrial dynamics and mitophagy in 2-methoxyestradiol-mediated osteosarcoma – and glial-cell death*, Przychodzen Paulina
S1.O6. *Alterations in plasma concentration and activity of superoxide dismutases, in context of: obesity and/or type 2 diabetes*, Lewandowski Łukasz

2nd SESSION

Moderators: Rene Kizek and Helena Moreira

11:15-11:45 Lecture of Prof. Rene Kizek (University of Brno, Czech Republic) *Nanomedicine for targeted treatment of tumor diseases* – abstract

11:50-12:50 Presentation of young scientists

S2.O1. *Effect of the surface charge on the structure of lysosome adsorbed on the gold surface*, Komorek Paulina
S2.O2. *Enhancement of antimicrobial photodynamic therapy using metallic nanoparticles*, Wanarska Ewelina
S2.O3. *Bile salts loaded Lipid Liquid Crystalline Nanoparticles for topical administration of natural antioxidants*, Fornasier Marco
S2.O4. *Functionalization of curdlan gel for effective antibiotic bonding*, Michalicha Anna
S2.O5. *Cytostatic effects of natural products on rhabdomyosarcoma – in vitro studies*, Kazimierczak Weronika

13:00-13:45 ***Flow Cytometry Webinar – Workshop*** (**Sysmex**, Joanna Rossowska and Helena Moreira)

3rd SESSION

Moderators: Joanna Tunikowska and Joanna Rossowska

13:50-14:20 Lecture of Dr. Joanna Tunikowska (Wrocław University of Environmental and Life Sciences, Poland) *Electrochemotherapy and lasers in surgical battle with tumors in dogs and cats* – abstract

14:25-15:25 Presentation of young scientists

S3.O1. *Determination of genetically modified dendritic cells' ability to survive, migrate to the lymph nodes and infiltrate the tumor tissue of MC38 colon carcinoma in in vitro and in vivo study*, Mierzejewska Jagoda

S3.O2. *Knowledge about coronary artery disease among Polish students – survey study*, Kanclerz Gabriela

S3.O3. *Development and evaluation of microsponge based topical gel for acne*, Sanjana Menezes

S3.O4. *Fluorescent polymeric nanocarriers of low cytotoxicity for two-photon bioimaging*, Nawrot Katarzyna

S3.O5. *Mixture of MMP-2, MLCK and NOS inhibitors induce cardioprotection against myocardial ischemia/reperfusion injury*, Krzywonos-Zawadzka Anna

S3.O6. *Personalization of pancreatic cancer treatment- electroporation, electrochemotherapy and calcium electroporation, pilot study*, Rudno-Rudzińska Julia

15:30-16:00 – Lecture of Dr. Tabassum Khan (Nanavati College of Pharmacy, India) *At the crossroad of drug discovery and delivery – PARP inhibitors and hollow metallic nanoparticles in cancer therapeutics* – abstract

10.10.2020 Saturday

4th SESSION

Moderators: Jolanta Saczko and Julita Kulbacka

9:00-10:00 Presentation of young scientists

S4.O1. *Thiazole based derivatives as sirtuins inhibitors. Molecular docking study*, Czyżnikowska Żaneta

S4.O2. *Fighting cancer with gravity*, Przystupski Dawid

S4.O3. *Combination of EP and PDT in melanoma treatment*, Szlasa Wojciech

S4.O4. *Pulsed Current Evaluation for Prediction of Tumor Permeabilization Rate*, Malyško Veronika

S4.O5. *A multifunctional nanoprobe based on polymeric nanocapsules loaded with quantum dots and lanthanide doped nanocrystals*, Antoniak Magda

S4.O6. *Light-induced in situ TEM microscopy revealing ultrastructural interactions in antimicrobial photodynamic therapy*, Andrzej Żak

5th SESSION

Webinar – 3D Bio-printing and holotompgraphic microscopy
10:10-10:40 Leader: Grzegorz Kaszyński (**Sygnis BioTechnolgies**)

6th SESSION

Moderators: Olga Pakhomova and Julita Kulbacka

10:45-11:15 Lecture of Prof. Olga Pakhomova (Old Dominion University, USA) *Calcium-mediated plasma membrane repair* – abstract

11:20-13:00 Presentation of young scientists
S6.O1. *Life support system in a remote Mars colony*, Piszko Paweł
S6.O2. *Changes in nuclear proteome associated with lamin in the Drosophila melanogaster model system after heat induction*, Pałka Marta
S6.O3. *Antiviral activity of extract of Ginkgo biloba (EGb) and its phytochemical constituents against herpesviruses HHV-1 and HHV-2*, Sochocka Marta
S6.O4. *Trimethylammonium 1-mercapto-1-carbadodecarnorate (TMA) – pharmaceutical precursor in BNCT method*, Wójciuk Karolina
S6.O5. *The structure of Nucleobindin-2 is regulated by divalent metal cations*, Skorupska Anna
S6.O6. *Stress affects the expression of 'major housekeeping' genes – is phosphorylation involved?* Tomczak Aleksandra
S6.O7. *Male infertility in context of oxidative stress: the analysis of Total Antioxidant Status and clusterin concentration in human seminal plasma – pilot study*, Janiszewska Ewa
S6.O8. *A different strategy to prevent protein adsorption*, Lupa Dawid
S6.O9. *Volatile compounds as a means of protecting bacterial contamination of cosmetics,* Surowiak Alicja K.
S6.O10. *Studies on the reactivity of human serum IgG and IgA antibodies with the bacterial OmpC protein as a potential diagnostic marker of humoral immuno-deficiency in children*, Naporowski Piotr

7th SESSION – *Molecular Biology Workshop*

Leader: Katarzyna Widerak (Syngen Biotech)
13:30-14:15 – MB webinar training

8th SESSION – *Molecular Dynamics Workshop*

Leader: Mounir Tarek
14:30-15:15 – MD webinar training

available all the time VIRTUAL POSTER SESSION

15:45 Announcement of the results for the best oral and poster presentations (awards predicted!) and closing ceremony, certificates

LECTURES

L 1.

Frontiers of Electroporation, from Mechanisms to Applications: Unravelling new key molecular level aspects using computational chemistry

Mounir Tarek

Centre National de La Recherche Scientifique (CNRS) Université de Lorraine, Nancy FRANCE

The application of short and intense electric pulses enables to transiently alter the properties of cell membranes, making them permeable to a wide range of chemical species. This phenomenon is routinely used in a range of medical applications as well in biotechnology and industrial processing. Few years ago, pioneering MD simulations have been conducted in order to model the effect of electric fields on membranes, providing perhaps the first molecular model of the electroporation process of lipid bilayers. Our knowledge however about all occurring processes is still sketchy. In this contribution we show how we harness the capabilities of computational resources and the predictive power of advanced atomistic and quantum level molecular dynamics techniques to decipher key steps in several physical and biophysical and chemical processes occurring at the cell membranes when these are subject to electric pulses used in Electroporation Based Technologies and Treatments.

L 2.

Nanomedicine for targeted treatmentof tumor diseases

Rene Kizek

Department of Human Pharmacology and Toxicology, University of Veterinary and Pharmaceutical Sciences Brno, Palackého třída 1946/1, 612 42 Brno, Czech Republic

Cancer is the second leading cause of death in developed countries. It is known that standard antitumor therapy has a number of serious adverse biological effects. One of these is the lack of selectivity for tumor tissue, resulting in significant side effects. The relatively low therapeutic concentration of the active compound often results in drug resistance and multi-resistance of tumor cells. Nanotransporters for targeted treatment are a modern and effective way of personalized approach. Carbon, gold, silver and other nanoparticles can be used as the basis of the nanotransporter. Nanoparticles can enter a cell independently of its type and functional group attached to the surface of the nanoparticle. Various *in vitro* and *in vivo* studies have shown that many functionalized nanoparticles are biocompatible. The physico-chemical properties of nanoparticles play a decisive role in their potential toxicity. For carbon nanoparticles, shorter and thicker nanotubes have been found to exhibit lower toxicity. Chemically functionalized carbon nanotubes (CNTs) are much better water-soluble and have greater stability in the physiological environment. Attempts to use CNTs to target multivalent ligands in cancer are increasing rapidly. In addition to passive targeting methods based on the enhanced permeability and retention (EPR) effect and the specific acidic environment in the tumor, strategies for actively targeting a selected tumor using ligands or antibodies that increase the specificity of the nanotransporter are also investigated. However, a protein corona plays a major role in the application of nanoparticles *in vivo*. A protein corona is a cluster of all proteins that can bind to nanoparticles. Protein corona formation is usually associated with a significant reduction in therapeutic potential. Albumin is the most abundant component of the protein corona. It has been shown that the composition of the protein corona depends on the structure and physico-chemical properties of the nanoparticles. However, the effect of surfactants on the structure of CNTs, on the composition and formation of the protein corona, has not yet been studied. In our experiments,the effect of the interaction of bovine serum albumin (BSA) and CNTs was studied. A completely unanswered question is the interaction of nanoparticles with thiol compounds such as low-molecular-weight glutathione or metallothionein. In addition to the above, in some malignant tumors we observe increased expression of albumin receptors (liver, gallbladder, but also breast cancer). This may be advantageous for nanoparticles with a protein corona. Research in this area of nanomedicine is completely open and will certainly bring many unexpected discoveries in the near future.

L 3.

Electrochemotherapy and lasers in surgical battle with tumors in dogs and cats

Joanna Tunikowska

Department of Surgery, Faculty of Veterinary Medicine, Wrocław University of Environmental and Life Sciences, Wroclaw, Poland

A successful treatment of patients with solid tumors consists of three components: complete tumor removal with tumor-free margins, identification and removal of tumor-positive lymph nodes, and removal of any local satellite tumor deposits (Holt et al., 2015). Therefore the vast majority of both human and veterinary oncologic patients require combination therapy: surgery and additional oncologic treatment. While the method of tumor excision depends mainly from surgeon the decision on additional oncologic treatment relies on patient general health status and tumor characteristics. Ten privately owned dogs and four cats presented to the Department of Surgery, Faculty of Veterinary Medicine WUELES with confirmed malignant tumors, were treated both laser surgery and electrochemotherapy. Laser CO2 is one of the most universal surgical lasers, allowing for precise excision and ablation of soft tissue with simultaneous sealing of small blood vessels, lymph vessels and nociceptors (Bartels, 2002). In addition to surgical treatment, local electrochemotherapy (ECT) was implemented before, during or after surgery. ECT is a local tumor treatment modality facilitating intracellular delivery of non-permeant chemotherapeutic drugs followed by the delivery of electrical pulses to the tumor. The advantages of ECT therapy are its simplicity, short duration of treatment sessions, low chemotherapeutic doses, and insignificant side effects with excellent functional and cosmetic effects (Tozon, Tamzali, & Cemažar, 2017). The combination of both laser CO_2 and ECT therapy can be successfully applied as a curative or palliative treatment also in animals with poor health status.

References:

[1] Bartels K. E. (2002). *Lasers in veterinary medicine – Where have we been, and where are we going?* Veterinary Clinics of North America -Small Animal Practice, 32(3), 495–515. https://doi.org/10.1016/S0195-5616(02)00002-5.

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L 4.

At the crossroad of drug discovery and delivery-PARP inhibitors and hollow metallic nanoparticles in cancer therapeutics

Tabassum Asif Khan

Department of Pharmaceutical Chemistry & QASVKM's Dr.Bhanuben Nanavati College of Pharmacy, Mumbai, India

Development of PARP inhibitors as cytotoxic leads in cancer therapeutics. Poly ADP-ribose polymerase-1 (PARP-1) is a nuclear enzyme essential to the repair of single strand DNA breaks via the base excision repair/single strand break repair pathway. Our lab has synthesized a series of heterocyclic compounds as potential PARP-1 inhibitors. Among these, quinazolinone scaffold based analogues have shown good activity. A series of twelve 2-styryl quinazolin-4(3H)-one analogues were synthesized by condensation of 2-methyl quinazolin-4(3H)-one with appropriate aromatic aldehydes.This scaffold was further used to synthesize a series of hybrids with pyrimidine analoguesThey were screened for cytotoxicity against MCF-7 cellsusing sulforhodamine B assay. The percentage yieldof synthesized compounds wasfoundto be in the range of 60 to 90%. The GI50 of 4-nitrophenyl ethan-1-ene quinazolin-4-(3H)-onewas found to be 8.1µg/mL comparable to standard Adriamycin. Flow cytometry study for the 4-nitrophenyl ethan-1-ene quinazolin-4-(3H)-one and 2-nitrophenyl ethan-1-ene quinazolin-4-(3H)-one indicated that the cells in early apoptosis were ~20% indicating caspase mediated death. Few of these along with the hybrid series werefound to be potent with good PARP-1 inhibitory activity.Design and synthesis of functionalized hollow metallic nanoparticlesin cancer therapeuticsMetallic nanostructures with hollow interiors are excellent agents for biomedical applications due to their Surface Plasmon Resonance (SPR) in Vis-NIR range. We report for the first time, an extensive study on the effect of (1) the addition sequence of stabilizer, (2) type of stabilizer, (3) the concentration of reducing agent (NaBH4) and(4) the reaction temperature on the SPR characteristic of glutathione-capped hollow silver nanoparticles (GSH-HAgNPs) using the sacrificial Ag_2O template. The photoablation of functionalized metallic nanoparticles (SPR at 531nm)using a 532 nm Nd:YAG 300 mW continuous wave (CW) laser led to a 5-6°C elevation in temperature above physiological temperaturewithin 15 minutessuggesting the use of GSHas hyperthermia-inducing agent. This study provides an evidence of the potential application of functionalized hollow metallic nanoparticles inbiomedicine, especially as drug delivery cargoes in cancer therapeutics.

L 5.

Calcium-mediated plasma membrane repair

Olga Pakhomova

Frank Reidy Research Center for Bioelectrics Old Dominion University, Norfolk, Virginia, USA

The plasma membrane isolates intracellular content from the environment and has the regulatory mechanisms to keep the cell homeostasis unperturbed. The membrane injuries destroy tight control over the cell metabolism and trigger a range of events varying from moderate stimulatory action to significant disorders in the homeostasis or even cell death. Membrane damages are a common threat to the life of the cells, especially for ones originating in the tissues exposed to mechanical or shear stress (muscles, lung, vasculature) or pore-forming toxins. In several medical applications (ultrasound or electroporation), impairing the plasma membrane barrier is a goal and used for drug delivery or tissue elimination. The cells have healing machinery for efficient repair of the membrane damages. Just a few seconds are needed to reseal a membrane pore or remove a membrane tear. Several different models are proposed for membrane restoration. All of them consider the calcium ion as the critical trigger for the repair response activation. The lecture aims at reviewing different cellular and molecular mechanisms of membrane repair, with emphasis on its relevance to disease.

ORAL PRESENTATIONS

Session 1, **O 1.**

Relationship between the concentration of IL-6, insulin activity, glycated haemoglobin in human blood and the development of type 2 diabetes and/or obesity

Magdalena Król[1], Iwona Urbanowicz[2], Łukasz Lewandowski[3], Marta Kepinska[3], Halina Milnerowicz[3]

[1]Students Scientific Association, Department of Biomedical Environmental Analyses, Faculty of Pharmacy, Wrocław Medical University; [2]Department of Medical Laboratory Diagnostics, Faculty of Pharmacy, Wrocław Medical University; [3]Department of Biomedical and Environmental Analyses, Faculty of Pharmacy, Wrocław Medical University

Background

Nowadays, obesity is a very serious problem in our society. Moreover, it may be associated with various metabolic diseases such as, type 2 diabetes (T2D). Many studies indicate the role of interleukine-6 (IL-6) in the pathophysiology of T2D. Increased level of IL-6 is an independent predictor of this disease and is considered to be involved in the development of inflammation, insulin resistance and β-cell dysfunction. On the other hand, recent research suggests that IL-6 has an anti-inflammatory role and improves glucose metabolism [1, 2].

Material and Methods

In this study, we analyzed relationship between the concentration of IL-6, insulin activity and other selected parameters, and the occurrence of T2D and/or obesity. These variables were assayed for human blood, obtained from 117 patients, of which 44 respondents belonged to obese without diabetic group and 23 respondents belonged to diabetic group. For comparison, all parameters were also measured in the control group, which consisted of 50 respondents.

The allocation to the obese group was based on the BMI value. In turn, we used the values of glycated haemoglobin as a marker of diabetes to assign to the diabetic group. Additional factors considered in this study were sex and exposition to cigarette smoke.

The IL-6 concentration and insulin activity in serum were tested using suitable ELISA kits.

Results

We observed significantly higher insulin levels in respondents with obesity compared to the control group, regardless of sex.

In addition, we noticed differences in IL-6 levels depending on whether the patient was obese or not. However, these differences were not statistically significant.

Interestingly, we didn't observe statistically significant higher levels of IL-6 in respondents suffering from T2D.

Discussion and conclusions

This research, as well as available literature, may show the important role of IL-6 and the other parameters, such as insulin, in the development of obesity and, consequently, contribute to the incidence of T2D [3]. Bastard et al. hypothesized that adipose tissue may play a role in the regulation of serum C-reactive protein concentration through IL-6 production [4].

Based on the available information relating to IL-6 its concentration seems to be a good indicator of activation of the inflammatory cascade and a predictor of subsequent organ dysfunction. However, our data, exactly as in Al-Shukaili et al. research, didn't show increased IL-6 levels in serum of patients

with diabetes [5]. Perhaps this was due to the small sample size. In any case, a better understanding of these mechanisms can support prevention and treatment of obesity and T2D.

References:

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Session 1, **O 2.**

Assessment of the stimulation level of the antitumor response by dendritic cells modified to overexpression of IL-12 and/or IL-18 *in vitro*

Katarzyna Węgierek[1], Jagoda Mierzejewska[1], Magdalena Geneja[1], Natalia Anger-Góra[1], Agnieszka Szczygieł[1], Joanna Rossowska[1], Elżbieta Pajtasz-Piasecka[1]

[1]*Ludwik Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wroclaw, Poland*

Background

Dendritic cells (DCs) – the most effective antigen presenting cells – are one of the therapeutic tools to enabling changes in the tumor microenvironment [1, 2]. The effect of IL-18 on the modulation of the immune response to a large extent depends on the presence of IL-12 in the environment. Owing to this fact, it seems reasonable to combine these elements in order to elaborate new strategies of cancer-treat-ment. DCs genetically modified to produce cytokine such as IL-12 and IL-18, may promote the activation of anti-tumor response [3, 4]. The main objective of these study was to determine the stimulation level of the antitumor response by DCs modified to simultaneous overex-pression of IL-12 and IL-18, stimulated with tumor antigens (TAg) in MC38 colon cancer immunotherapy.

Material and Methods

Dendritic cells were generated from bone marrow (BM) collected from healthy C57BL/6 mice. After seven days of culture, BM-DCs were co-transduced with *il12* and *il18* genes and/or treated with MC38 tumor lysate (TAg). On the 9^{th}, 11^{th}, 13^{th} and 15^{th} days of culture, cytokine gene expression (real-time PCR), cytokine production (ELISA) and expression of DCs surface markers (flow cytometry) were evaluated. In addition, changes in percentage of spleen cell populations ($CD4^+$, $CD8^+$, NK cells, $CD107a^+$) from 5-days mixed cultures BM-DC and cytotoxic activity of splenocytes (flow cytometry) were examined.

Results

The high level of *il12* and *il18* gene expression and production of these cytokines by transduced BM-DCs were observed. Moreover, significant changes in the expression of co-stimulatory molecules depending on the type of transduction and length of culture were determined. Furthermore, splenocytes preincubated with BM-DCs transduced for production of cytokines and stimulated with TAg in mixed culture revealed an increase in tumor antigen-specific cytotoxicity. In addition, an increase

in the percentage of $CD4^+$ T cells, NK cells and $CD107a^+$ in mixed culture of splenocytes and BMDCs transduced for cytokine production were observed.

Discussion and conclusions

The received results suggest that DCs transduced with *il12* and *il18* genes, additionally stimulated with tumor antigens are able to trigger an antitumor response *in vitro* and *ex vivo*. This suggests their potential in generation of various DC-based vaccines for anti-tumor immumotherapy.

This study was funded by National Science Centre, Poland (projects no 2015/17/N/NZ4/02834, 2017/27/B/NZ6/02702).

References:

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[2] Palucka K., Banchereau J. *Nat Rev Cancer*, vol. 12: 265-277; 2012.
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Session 1, **O 3.**

Development of new polymeric materials with the incorporated API for potential application in solid dosage forms formulation using 3D printing technology

Marta Kozakiewicz[1], Maciej Gajda[1], Adrianna Złocińska[2], Bożena Karolewicz[1], Karol P. Nartowski[1]

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Background

3D printing is a potential technology suitable for overcoming limitations for development and manufacturing of personalized medicines. The biggest challenge for wide application of 3DP technologies in personalised medicines is lack of robust 'ready to use' materials of pharmaceutical quality, which could easily be used in this process [1].

The aim of this work was to synthesize new polymeric materials with incorporated API (Active Pharmaceutical Ingredient), which can be used for FDM 3D printing method (Fused Deposition Modeling).

In this work we design drug/polymer/plasticizer blends, which after processing using hot melt extrusion (HME) formed plastic filaments "ready to use" in FDM based 3DP methods.

Material and Methods

Amlodipine (AMLO) and hydrochlorothiazide (HTZ) (Polaura, Olsztyn Poland), simvastatin (SIM) and nicotinamide (NIC) (Sigma Aldrich), polymers (HPMCAS 126 AQOAT® type AS-HF and AS-LF Shin-Etsu), and obtained filaments were tested for thermal stability prior HME processing using thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC). The structural changes of the APIs that might occur during manufacturing process were investigated using. X-ray powder diffraction (PXRD) and Fourier Transform Infrared Spectroscopy (FTIR). The filaments were prepared using twin screw hot melt extrusion (Thermo Scientific Process 11 Extruder), and a series of placebo tablets and single-component tablets with simvastatin were printed using the 3D printer "Builder Premium".

Results

Two types of placebo filaments were produced from HPMCAS AS-HF (I) and AS-LF (II) polymers and five different types of filaments with incorporated therapeutic substances: SIM+ HPMCAS HF(A), SIM + AMLO + HPMCAS HF (B), AMLO + HTZ + HPMCAS LF (C), AMLO + HTZ + Crospovidone + HPMCAS LF (D), Cocrystal HTZ:NIC + HPMCAS LF (E). Two series of tablets using obtained filament were successfully printed: placebo HPMCAS HF series and single-component tablets with SIM.

The improvement of the plasticity of the fabricated filaments through the addition of plasticizer enabled 3D printing process. Modification of melting temperature by production a co-crystal to adapt thermal properties to the process window was presented [2].

Discussion and conclusions

Thermal and mechanical properties (i.e. thermal stability and glass transition) of the materials are the key parameters enabling the use of polymers and APIs in the process of hot melt extrusion and 3D printing by the FDM method. The plasticity of a filament with incorporated API depends on the phase of the drug in the formed filament (i.e. crystalline or amorphous) which has been proven in our work. The occurrence of the API in an amorphous state caused increase of elasticity of filaments, whereas the presence of drug crystals incorporated in the filament increased the brittleness and fragility of the extrudates.

The obtained results confirm the possibility of producing pharmaceutical-quality fila-ments using hot extrusion and their subsequent use in the manufacturing of solid oral dosage forms using FDM 3D printing technology.

This research was funded by the Ministry of Science and Higher Education, Poland through the National Fund for Scientific Research (Grant No. ST.D 190.18.001).

References:

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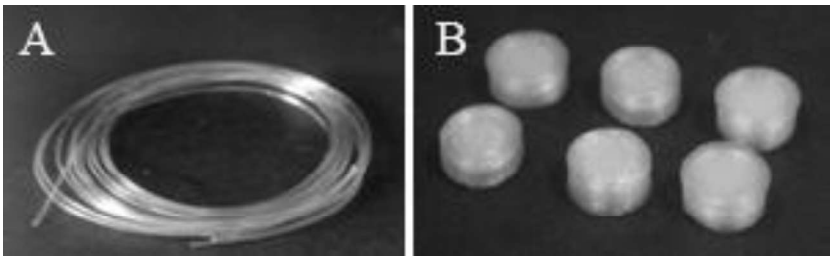

Fig.1 (A) Filament with incorporated simvastatin. (B) Printed tablets with simvastatin

Session 1, **O 4.**

Antitumor activity of therapy composed of methotrexate nanoconjugate and dendritic cell-based vaccines and its influence on local and systemic antitumor immune response

Agnieszka Szczygiel[1], Katarzyna Węgierek[1], Natalia Anger-Góra[1], Jagoda Mierzejewska[1], Tomasz Goszczyński[1], Marta Świtalska[1], Joanna Rossowska[1], Elżbieta Pajtasz-Piasecka[1]

[1]*Ludwik Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wroclaw, Poland*

Background

The conventional form of chemotherapy, including methotrexate, are related with overall acute toxicity to healthy cells, rapid elimination of chemotherapeutics from the body and low specificity towards target cancer cells. Thus, it was challenging to design a chemotherapeutic-carrier system overcoming these difficulties. The HES-MTX nanoconjugate was obtained by covalent coupling of well-known therapeutic compounds – methotrexate as anticancer agent and hydroxyethyl starch as high-molecular carrier [1]. The main advantage of HES-MTX nanoconjugate over the MTX in free form is prolonged half-time in plasma and specific biodistribution, which is realized mainly by interacting with folate receptors alpha (FRα) overexpressed on tumor cells or through enhanced vascular permeability and retention effect (EPR).

The main objective of the study was to determine whether HES-MTX nanoconjugate can modulate the systemic antitumor immune response and affect the changes in the landscape of immune cells infiltrating into tissue of murine colon carcinoma MC38. This, in turn, should contribute to the creation of efficient immune response against growing tumor by DC-based vaccines multiple injected after chemotherapy administration.

Material and Methods

Female C57BL/6 mice (8-10 weekd old) were subcutaneously inoculated with MC38 cells. In course of chemotherapy, on the 14th day of experiment, mice received intraveno-usly MTX or HES-MTX (20 mg/kg b.m.) and 3 days later (17th day of experiment) 5 mice from each group were sacrificed and tumors and spleens were dissected. Mice received chemotherapy on 14^{th} day of experiment and on the 17^{th}, 24^{th} and 31^{st} day of experiment tumor antigen-stimulated dendritic cell based vaccines (DC/TAg) were applied peritu-morally. In group of mice from untreated and chemotherapy treated groups tumor nodules and spleens were dissected on the 31st day of experiment, and from DC/TAg-receiving groups group tumor nodules and spleens were dissected on the 35^{th} day of experiment (3-5 mice per group). Tumor cells and spleen cells were analysed by multipa-rameter flow cytometry according to the procedure described previously [2]. During analyses the percentage of lymphoid as well as determination of their activity, and percentage of myeloid cells as well as identification of macrophage stage polari-zation through expression of CD206 intra-cellular antigen were evaluated. Moreover, determination the ability of splenocytes obtained from treated MC38 bearing-mice to generate efficient anti-tumor response was conducted. After five-days restimu-lation of spleen cells with mitomycin C-treated MC38 cells, the phenotype and CD107a degranulation assay and cytotoxic activity of splc against MC38 cells were determined.

Results
Three days after HES-MTX administration the enlargement of CTLs population size CD8+ T cells, NK, NKT cells) in spleen and tumor tissue, as well as in reduction of the population size of immune cells with suppressor activity (Tregs, TAMs, Mfs and M2-type macrophages) in tumors. Moreover, after restimulation, spleen cells obtained from HES-MTX-treated mice revealed higher percentage of CD8+ T cells and the highest cytotoxic activity of splenocytes against tumor cells. This contributed to creation of favourable environment necessary to promote the development of anti-tumor immune response by dendritic cell-based immuno-therapy, which was reflected especially in delay of tumor growth.

Discussion and conclusions
The administration of HES-MTX influenced the systemic immune response and enhanced the cytotoxic activity of splenic CTLs, but also changed the hostile landscape of immune cells infiltrating into tumor tissue. This contributed to creation of favourable environment necessary to promote the development of anti-tumor immune response by dendritic cell-based immunotherapy, which was reflected especially in delay of tumor growth.

The study was funded by National Science Centre (project no. 2015/19/N/NZ6/02908 and 2017/27/B/NZ6/02702).

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Session 1, **O 5.**

Regulation of mitochondrial dynamics and mitophagy in 2-methoxyestradiol-mediated osteosarcoma- and glial-cell death

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Background
Osteosarcoma (OS) is one of the most malignant tumors of childhood and adolescence [1], whereas gliomas are comparatively rare human cancers of the central nervous system (CNS) [2]. Research on mitochondrial dynamics (fusion/fission), mitochondrial biogenesis and mitophagy have received much attention in last few years, as they are crucial for understanding of many biological processes, including cancer cell death [1]. Specifically, it was shown that increasing the cytoplasmic dynamin-related protein

1 (Drp1) expression activates mitochondrial fission, which resulted in BAX activation and downstream intrinsic apoptosis, effectively inhibiting osteosarcoma growth [1]. Notably, Drp1 mediates also activation of mitophagy, specific type of autophagy [3].

2-methoxyestradiol (2-ME) is a physiological derivative of 17β-estradiol that possesses anticancer activities, confirmed by *in vitro* and *in vivo* studies [4,5].

Previously, we evidenced that from mechanistic point of view, one of anticancer mode of action of 2-ME is a selective induction of overexpression of neuronal nitric oxide synthase (nNOS) and enzyme nuclear translocation. Nuclear translocation of nNOS results in local nitro-oxidative stress leading to DNA damage in cancer cells [6].

Material and Methods

We used highly metastatic osteosarcoma 143B and glial SW 1088 cell lines in the study and cellular biology methods. In order to search for signaling pathways of 2-ME we used flow cytometry, stopped flow, electron microscopy and confocal microscopy techniques.

Results

Herein, we present that nuclear generation of nitric oxide leads to inhibition of mitochondrial biogenesis in highly metastatic osteosarcoma 143B cells. We further investtigated whether 2-ME may be a regulator of mitochondria dynamics in osteosarcoma cell death model. We demonstrated an important role of mitochondrial division in efficacy of 2-ME. We also established that the mitochondrial division inhibitor 1, MDIVI-1 induces cell death and sensitizes the osteosarcoma cells to 2-ME-meditated cell death.

Discussion and conclusions

Herein, we established that, MDIVI-1 induces cell death and sensitizes the osteosarcoma cells to 2-ME-meditated cell death. Previously, it was reported that MDIVI-1 enhanced cisplatin induced cytotoxicity in association with increased oxidative stress [7]. Indeed, it was previously well established that MDIVI-1 downregulates the Drp1 [8]. We observed that 2-ME upregulated Drp1 level. Due to induction of nitric oxide derivatives, we suggest that 2-ME-mediated *S*-nitrosylation of Drp1 may be one of the key regulatory adaptations for mitochondrial dynamics in cancer. For further investigation we will explore role of mitochondrial dynamics in osteosarcoma progression and in glioma.

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Session 1, **O 6.**

Alterations in plasma concentration and activity of superoxide dismutases, in context of: obesity and/or type 2 diabetes

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Background

Superoxide dismutases (SODs) are a family of enzymatic antioxidants. Each SOD isozyme originates from different cellular compartments: cytosol (SOD1), mitochondria (SOD2) and extracellular fluid/cell membranes (SOD3). Lately, and increasing level of attention has been drawn to the activity of SODs in context of insulin resistance, as SODs have been shown to protect pancreatic beta cells from oxidative stress. This research aims to point out the possible effect of obesity and/or diabetes, and other selected factors, on the concentration and/or activity of SODs.

Material and Methods

This research focuses on two, often co-existing, diseases – obesity and type 2 diabetes. The research aimed to examine the concentration of SODs: SOD1, SOD2, SOD3, and their corresponding activity (total SOD activity, Cu,Zn-SOD activity, Mn-SOD activity), and compare these values to total antioxidative capacity (TAC) and the concentration of malondialdehyde (MDA), a lipid peroxidation product. These variables were assayed for plasma/serum, obtained from 117 individuals, of which: 50, 44, and 23 belonged to: control group, obese, non-diabetic group, and diabetic group, respectively.

Xanthine oxidase method was used to measure total SOD activity in plasma. Potassium cyanide, a selective Cu,Zn-SOD inhibitor, was used in this method, to determine both: Cu,Zn-SOD and Mn-SOD activity. Plasma concentration of: SOD1, SOD2, SOD3 were assayed for with use of ELISA kits. TAC was assayed for in uric acid equivalents, with use of copper reduction method. MDA concentration was assayed for with use of thiobarbituric acid method. In this research, the concentration and activity of SODs were presented, and analysed, in different units, found in the literature.

Additional categorical factors analysed in this research, except for obesity and type 2 diabetes, were: sex and exposition to cigarette smoke. The exposition to cigarette smoke was evaluated with use of an univariate logistic regression model, based on the serum concentration of nicotine metabolite – cotinine (assayed for with use of an ELISA kit).

Results

Regardless of sex and exposition to cigarette smoke, the diabetic groups (non-obese and obese) showed a markedly increased concentration of SOD1. An increase in Cu,Zn-SOD activity was found in diabetic groups, compared to non-diabetic. However, when displayed in [U/mg SOD1+SOD3], the values of Cu,Zn-SOD showed a decreasing trend (control > obese, non-diabetic > diabetic, non-obese > diabetic, obese). Similar, but less pronounced differences were found in values of total SOD activity, but not in Mn-SOD activity. Obese individuals were characterized of higher TAC values than the non-obese.

Regardless of obesity and/or type 2 diabetes, males were characterized of significantly higher SOD1 concentration. TAC values were also higher in males, compared to females, although this difference was not significant in the obese, non-diabetic group. However, no significant difference was observer in other analysed variables.

There were no significant differences between the individuals exposed to cigarette smoke, in all of the analysed variables. Interestingly, this lack of dependency was observed also in concentration of MDA.

Discussion and conclusions

This research featured both the values of different SODs, in plasma, simultaneously, in the same dataset. To our knowledge, not many research papers cover such data, thus – the discussion might prove difficult. However, several research showed a significant positive correlation of SOD activity with BMI, or insulin [1, 2]. However, some research show no such dependence [3].

Several research show higher SOD activity in: obese, compared to non-obese [4], and type 2 diabetic individuals, compared to non-diabetic[5]. Interestingly, to our knowledge, an increase in plasma SOD1 concentration was not observed before in the diabetic group, although an increase in SOD3 concentration has been observed.

The results of this research may indicate that in case of obesity and type 2 diabetes, the organism may adapt to increased oxidative stress, with increase in TAC and Cu,Zn-SOD activity.

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Session 2, **O 1.**

Effect of the surface charge on the structure of lysosome adsorbed on the gold surface

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Background

The neurodegeneration process is associated with proteins which transform from their native form to partially changed structure and finally to aggregates which accumulate in affected tissues. The progression of neurodegeneration is associated with the growth of protein aggregated to fibrils. It is preceded by initial changes in the secondary structure of the protein. Adsorption of protiens on the liquid/solid interface is known to be a fundamental phenomenon that can cause protein folding disorders. An in-depth investigation of the effect of the surface-protein interactions on its secondary structure could provide new insights into the aggregation mechanisms. The aggregation of proteins in bulk solution has been extensively studied while in situ studies of secondary structure of proteins in the adsorbed state are stil not well understood.

Material and Methods
In the studies presented here, lysozyme (L6876, Sigma Aldrich) from chicken egg white was used. It was used without further purification. Lysozyme adsorption on the gold surface as a function of the pH the electrolyte solution wass monitored by Multi-Parametric Surface Plasmon Resonance (MP-SPR) and Quartz Crystal Microbalance with Dissipation Monitoring (QCM-D). The secondary structure of lysozyme in solution was analyzed by Circular Dichroism (CD), while changes in the protein structure in the adsorbed state were investigated using Polarisation Modulation-Infrared Reflection Absorption Spectroscopy (PM-IRRAS).

Results
In the presented work, we selected hen egg lysozyme and gold surface to study factors having a major impact on the mechanism of protein adsorption and the conformation of the adsorbed protein on the solid surface. By using MP-SPR and QCM-D the nature of the interactions and related amount of adsorbed protein were investigated and allowed us to determine the orientation of molecules on the gold surface. It was also confirmed that the rearrangement of molecules caused by changes in surface coverage displays themselves in the macroscopic properties of formed layers. Comparison of CD and PM-IRRAS results in solution and in the adsorbed state, respectively showed changes in the secondary structures of lysozyme. The adsorption of lysozyme was studied as a function of surface charge on the protein (varions of pH) and surface charge density on the metal surface. The protein films were investigated using in situ PM-IRRAS. Electrostatic attraction between the metal surface and lysozyme facilitate the protein accumulation on the Au surface. The results of our studies show that in the protein misfolding process, the appreance of disordered structures is first observed.

Discussion and conclusions
The presented data unambiguously indicate that protein surface coverage and charge at the surface of molecules can influence the properties and structure of molecules coming in contanct with solid surfaces. The surface charge has a significant impact on the conformation and structure of proteins adsorbing on solid substrates. However, the mechanism of changes in the conformation of lysozyme at the surface is still not completely understood. These results are particularly relevant for understanding the process of protein aggregation.

Acknowledgements
This work was partially supported by project NCN OPUS 2016/23/B/ST5/02788, InterDokMed POWR.03.02.00-00-I013/16 and NAWA PPN/BIL/2018/1/00103.

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Session 2, **O 2.**

Enhancement of antimicrobial photodynamic therapy using metallic nanoparticles

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Background

In XXI century bacterial infections are global problem. The antibiotic therapy is the most common way of treatment. The ESKAPE acronym describes bacteria, that do not undergo antibiotic therapy: *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa* and *Enterobacter* [1]. Antimicrobial photodynamic therapy, based on using non toxic dyes (photosensitizers) and special light in present of oxygen, turned to be new approach to kill resistant bacteria by generation of reactive species of oxygen [2]. Nanotechnology, as a field of study which deals with the creation and manipulation of metallic nanoparticles, allows to create of new generation photosensitizers, which are able enhance process of killness [3].

Material and Methods

In this study the impact of various concentrations of methylene blue and gold nanoparticles was tested against *Staphylococcus aureus*, using luminescence test and series of dilution method. Then bactericidal effect of photodynamic inactivation with LED light irradiation was tested using series of dilution method. Gold nanoparticles was used as enhancement agents of photodynamic therapy, using previous mentioned method.

Results

Methylene blue used as photosensitizer caused 92% mortality of *Staphylococcus aureus* cells after 30 minutes of irradiation. Gold nanoparticles used as enhancement agents caused 96% mortality of *S.aureus* cells.

Discussion and conclusions

Process of photodynamic therapy is widely described. Tawfik et al. described similar results using laser light irradiation (660 nm) receiving 97% mortality of *S.aureus* using methylene blue and gold nanoparticles as enhacement agents [4]. Antimicrobial photo-dynamic therapy using methylene blue as photosensitizer caused bactericidal effect. The presence of gold nanoparticles enhanced the antimicrobial effect of therapy.

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Session 2, **O 3.**

Bile salts loaded Lipid Liquid Crystalline Nanoparticles for topical administration of natural antioxidants

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Background

Bile Salts (BSs) are natural surfactants deputed to lipid digestion *in vivo*. The behaviour of these biologically important compounds has been well-studied to understand many complicated biological systems such as drug absorption in the small intestine, lipid bilayer mobility and phospholipid-bile salts interactions [1-3]. Nevertheless, their potential in nanomedicine for topical administration has not been fully studied. Indeed, the first layer of the skin, *stratum corneum,* represents the first obstacle in this kind of application due to its very dense and rich lipid matrix in which the keratinocytes are embedded. Lipid formulations are able to fluidify stratum corneum, allowing a drug to diffuse through it [4]. Therefore, we studied the effect of BSs on Lipid Liquid Crystalline Nanoparticles structure and their penetration properties *in vitro* for the delivery of natural antioxidants.

Material and Methods

Using monoolein as building block to prepare NPs in water and Pluronic F108 as stabilizer, the effect of three bile salts (Sodium Cholate, Deoxycholate and Taurocholate) on the NPs physicochemical features was studied. After an initial screening, the best candidates in terms of colloidal stability were loaded with catechin, a natural antioxidant, and their penetration properties on skin were evaluated *in vitro*.

Results

SAXS data showed how BS affect monoolein self-assembly leading the system from a cubic phase (cubosomes) to vesicular structures. When oleic acid is added to the mixture, a phase transition from unilamellar vesicles to hexosomes is highlighted also by Cryo-Tem (Fig 1.).

DLS, ELS and SAXS showed that Taurocholate gave the most stable formulations in comparison with Cholate and Deoxycholate. Catechin was encapsulated in hexosomes, BS-loaded hexosomes and vesicles and these three formulations were compared *in vitro* to understand how the structure and BSs can affect the penetration properties.

Discussion and conclusions

Among the BSs studied, Taurocholate is the most hydrophilic: the presence of a negative head-group ($-SO_3^-$) at the lipid/water interface increased the NPs Zeta potentials, giving a higher colloidal stability to the aggregates.

By tuning oleic acid and BS concentration, and therefore the curvature of the interface, it was possible to obtain vesicles or hexosomes. The encapsulation of catechin did not affect significantly the NPs physico-chemical features. The penetration tests *in vitro* showed that the presence of BS in the NPs can enhance the penetration properties giving almost 26 % of catechin in the first layers of the skin (*stratum corneum* + *epidermis*) in comparison with the hexosomes and vesicles without BSs.

In conclusion, we investigate the effect of BSs on the physico-chemical parameters of NPs made with monoolein and stabilized by Pluronic F108. Moreover, we showed that BSs are able to enhance significantly NPs penetration properties on skin for topical delivery of natural antioxidants.

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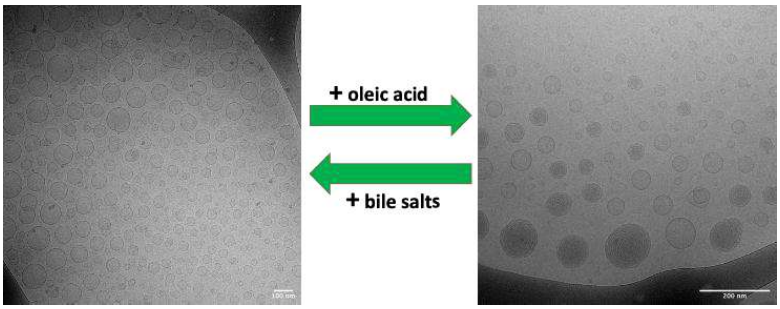

Fig. 1. Cryo-TEM images of the samples containing Taurocholate

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Session 2, **O 4.**

Functionalization of curdlan gel for effective antibiotic bonding

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Background

Currently, various synthetic and natural polymers are used for regenerative medicine. One of natural polymers of high biomedical potential is curdlan which forms hydrogel of high strength and elasticity. Also, it exhibits high water-absorption and retention capacity, therefore may be useful in fabrication of e.g. hydrogel dressing materials or other biomaterial resources (Cai and Zhang, 2017).

Coupling of therapeutical substances to curdlan matrix could increase its biological potential. However, the disadvantage of curdlan is the lack of active groups for modifications, such as amino and carboxyl groups which could enable binding of antibacterial or anti-inflammatory agents. Strategies used for curdlan functionalization include, among others, sulfonation, amination, oxidation, esterification or phosphorylation (Cai and Zhang, 2017; Zhang and Edgar, 2014) but they typically lead to curdlan solubilization and loss of beneficial physic-chemical properties. Recently, catecholamine polymers were used for functionalization of different matrices (Lee et al., 2007). Therefore, catecholamine derivative was used to functionalize the curdlan matrix by formation of strongly adhesive ad-layer. Such functionalized matrix was used as a template for antibiotic (gentamicin) immobilization. Some of the properties of such produced drug-loaded hydrogel were tested, including antibacterial activity against three reference bacterial strains and drug release profile.

Material and Methods

8% (w/v) curdlan suspension gelled at 93°C was used as a matrix. Functionalization of polysaccharide matrix was performed by hydroxytyramine polymerization from 2 mg/ml (or 4 mg/ml) solution for 24 h at 25°C. Monomer (hydroxytyramine) was added to curdlan matrix before its gelation (sample 2-D-BG and 4-D-BG) or after (2-D-AG). Non-attached polymer was eluted from the matrix in DI water (as monitored by UV-VIS spectrophotometry). Gentamicin was immobilized on functionalized matrices during their incubation in 1 mg/ml aqueous drug solution with and without activation with 5% glutaraldehyde. Drug release from the matrices was performed by incubation

of curdlan samples in PBS pH 7.4 at 37°C, with daily exchange of buffer. In collected samples, gentamicin was estimated after derivatization by phthaldialdehyde (Ginalska et al., 2004). Antibacterial activity of functionalized curdlan was evaluated by indirect method, in extracts collected in similar way as in drug release test (PBS was replaced by Mueller-Hinton Broth). The extracts were inoculated by *S. aureus* ATCC 25923, *S. epidermidis* ATCC 12228 and *E. coli* ATCC 25922 reference strains and allowed to grow at 37°C for 24h. Then the bacterial growth was estimated as optical density at 660 nm in Synergy H4 plate reader (Biotec, USA). The water uptake was evaluated during 72 hours incubation in water, as a function of wet weight increase.

Results

The catecholamine layer on matrix was regular, without noticeable precipitates and colour differences. Larger amount of gentamicin was immobilized to the curdlan sample which was modified by hydroxytyramine added before polysaccharide gelation (2-D-BG) in comparison with added after gelation (2-D-AG). 2-fold increase of amount of hydroxytyramine monomer for curdlan functionalization caused the slight (by approx. 25%) increase of drug immobilization. Activation with glutaraldehyde did not affect the binding capacity of the drug to functionalized curdlan matrix. The results of the experiment of gentamicin release to PBS solution indicated that the 4-D-BG sample the most effectively released the drug. Determination of the antibacterial properties of drug-loaded hydroxytyramine-functionalized curdlan samples showed that these matrices were able to protect the culture medium against bacterial infection for a long time. The weakest protection was observed against *E. coli* strain while against S. *epidermidis* and *S. aureus* strains this protection lasted for a minimum 28 days. All samples absorbed water in 700-900 % of its initial weight.

Discussion and conclusions

Modification of curdlan matrix using polyhydroxytyramine allows efficient binding and release of the antibiotic and protects the matrix against bacterial infection. The matrix modified in this way can be used in future to create dressing materials for the treatment of postoperative wounds and to protect them against infection.

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Session 2, **O 5.**

Cytostatic effects of natural products on rhabdomyosarcoma – *in vitro* studies

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Rhabdomyosarcoma (RMS) is the most common soft tissue sarcoma of the childhood. There are 3 types of RMS based on histological diagnosis: pleomorphic rhabdomyosarcoma (PRMS), embryonal rhabdomyosarcoma (ERMS) and alveoral rhabdomyosarcoma (ARMS). Depending on the type of the cancer, it is located in soft tissues of the extremities, neck, eye area or genitourinary organs. The treatment of RMS invilves the radiation therapy and chemo-therapy in combination with tumor resection [1, 2] and vincristine are currently approved by the FDA (Food and Drug Administration) for the treatment of RMS [3].

The aim of our research is to find new potential cytotoxic substances against ERMS. We put emphasis on natural products such as betulinic acid, biochanine and jasplaki-nolide. The results of the MTT assay showed that all tested substances are cytotoxic to ERMS after 24 and 72 hours incubation, although the concentrations of the cytotoxic substances differ from each other. At the same time, the analysis of the effect of these substances on fibroblasts was undertaken as a control group for ERMS, using the same concentrations of the substances. Each substance is cytotoxic to fibroblasts, but the fibroblasts' survival rate is different compared to ERMS. Optimal ERMS cytotoxicity parameters will be selected from the collected results, which will be used for further *in vivo* studies using the zebrafish model.

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Session 3, **O 1.**

Determination of genetically modified dendritic cells' ability to survive, migrate to the lymph nodes and infiltrate the tumor tissue of MC38 colon carcinoma in *in vitro* and *in vivo* study

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Background

Dendritic cells (DCs) play crucial role in regulation of the immune response. They are involved in promoting the humoral response as well as cellular response [1]. Because of their huge potential, DCs are a promising tool in fight against cancer. For induction of specific immune response, research focusing on the application of DCs led to the development of methods for their *ex vivo* activation with tumor antigens (TAg). Such stimulated DCs used as a vaccine, increase the probability of recognition of the heterogeneity in cancer cell populations by the host immune system and stimulation of CD^{4+} and CD^{8+} T cells [1,2]. However, the administration of DC-based vaccines leads only to temporary reduction of tumor growth. For this reason, their effect is enhanced by delivery such cytokines as IL-12 and/or IL-18.

The aim of the study was to determine genetically modified dendritic cells survival and their ability to migrate to the lymph nodes and to infiltrate the tumor tissue of MC38 colon carcinoma.

Material and Methods

The C57BL/6 mice were inoculated subcutaneously (s.c.) in a right flank with MC38 colon carcinoma cells ($1.1x10^6$ cells/mice, day 0). On the 15^{th} day, mice with established tumors were administered peritumorally (p.t.) with BM-DCs genetically modified to IL-12 and IL-18 coproduction, stimulated with MC38 tumor cell lysate ($2x10^6$ cells/mice) or control cells. In order to identify transduced cells in lymph nodes and tumor tissue, staining with a fluorescent dye CFDA-SE were performed. Lymphoid organs and tumor tissue were collected from mice on the 3^{rd}, 5^{th} and 7^{th} day after a single administration of cell vaccines. In addition, the proliferation rate and survival time of vaccine cells *in vitro* were evaluated.

Results

In the first stage of research, CFDA-SE vaccine cell staining conditions were optimized. In the next stage of the *in vitro* study, the proliferation rate and survival time of dendritic cells on the 3^{rd}, 5^{th} and 7^{th} after a single administration of vaccines were determined and were depended on the type of gene transduction. Transduced cells, stained with a fluorescent dye CFDA-SE, were observed in lymph nodes and tumor tissue. The numbers of CDFA-SE cells identify in lymph nodes and tumor tissue were depended on the type of applied vaccine cells and the duration of the experiment.

Discussion and conclusion

The obtained results suggest that DCs genetically modified to overproduction of IL-12 and/or IL-18 are able to survive, proliferate and migrate to the lymph nodes for 7 days in *in vitro* and *in vivo* conditions and they can be effective tools in anti-cancer therapy.

This study was funded by National Science Centre, Poland (projects no 2015/17/N/NZ4/02834, 2017/27/B/NZ6/02702).

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Session 3, **O 2.**

Knowledge about coronary artery disease among Polish students – survey study

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Background

Coronary artery disease (CAD) is one of the most common cause of death in Poland. Nevertheless, the level of knowledge about this pathology, risk factors and complication seems to be insufficient in our society. The aim of our study was to investigate the level of knowledge about CAD among Polish students.

Material and Methods

We conducted a survey study by internet questionnaire. Interviewees were 173

students of Polish universities. The questionnaire was prepared in accordance to second version of Coronary Artery Disease Education Questionnaire (CADE-Q II). There were 31 questions that assess students' literacy, in each four options to choose: right answer (for 2 points), half-right answer (1 point), wrong and „I don't know" answer – both marked as 0 points. The maximum overall score of the test was 62 points. Statistical analyses were performed with the Statistica 13.1 (StatSoft, Statistica 13.1, Tulsa, Oklahoma, USA) software. Continuous variables are expressed as a mean ± standard deviation or median (interquartile range) and categorical variables as a number (percentage). Continuous variables were first checked for normal distribution by the Shapiro-Wilk test and then were compared by Student's t-test or U-Mann Whitney test if distribution was normal or different than normal, respectively. Categorical variables were analyzed using the chi-squared test or Fisher's exact test. All independent variables associated ($P < 0.2$) with the score of questionnaire in an univariate model and not correlated with another independent variable were then included in the multivariate linear regression analysis to determine the score of survey. Two-sided P-value of less than 0.05 was considered statistically significant.

Results

We collected answers from 173 participants. Among them, there were 60 men (34.7%). The mean age of contributors was 22.0 (21.0-22.0) and the mean overall result of the survey was 48.0 points (44.0-52.0). In the questionnaire, 118 participants declared the contact with cardiovascular diseases (CVD) that was defined as their own illness or their family members or friends being affected. Surprisingly, in direct comparison of both groups – the students who had contact with CVD and who not had, there were no significant differences in terms of gender, age, the place of residence and the sum of the survey. The trend to higher self-assessment of knowledge was observed in contributors who had contact with CVD ($P=0.06$). By multivariable analysis, the younger age ($\beta=-0.87$, $P=0.001$; β – standardized linear regression coefficient) and higher self-assessment of knowledge ($\beta=2.58$, $P=<0.001$) was independently associated with higher overall survey score.

Discussion and conclusions

The knowledge about CAD in polish students may be considered as insufficient, Unfortunately, the personal contact with CVD did not correlate with higher CAD literacy. Further CAD awareness campaigns are necessary to gain adequate knowledge about CAD in Polish students.

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Session 3, **O 3.**

Development and evaluation of microsponge based topical gel for acne

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Background

Acne vulgaris is a common chronic skin condition caused due to inflammation of the pilosebeaceous unit – associated with the hair follicles and sebaceous glands. Acne affects approximately 80% of teenagers and is characterized by tender inflammatory papules and nodules mainly scattered on the face, neck, chest and upper back. The treatment of acne includes use of retinoids, antibiotics, herbal products, anti-androgens, vitamins and miscellaneous (salicylic acid and benzoyl peroxide) used alone or in combination.

Novel drug delivery systems have been used to optimize the delivery of diverse therapeutic agents [1]. Controlled release of the drug from the formulation into the epidermis such that the drug remains primarily localized with only a restricted amount of drug entering the systemic circulation is a means of controlling the side effects. There is need to maximize the time for the active ingredient to remain on the skin while minimizing percutaneous transdermal absorption. Recently, several reports are published describing various drug loaded microsponge formulations of drugs for topical application. Hence, an attempt was made to develop a microsponge gel containing adapalene and azelaic acid for acne. The objective of this study was to prepare microsponges using double emulsification solvent evaporation method, study the effect of various formulation parameters like drug: polymer ratio, solvent: polymer ratio, stirring rate and emulsifier concentrations on the physical characteristics of the microsponges, and study its efficacy using *in vitro* and *ex vivo* methods [2].

Material and Methods

Azelaic acid and adapalene were selected as model drugs. The microsponges were prepared by w/o/w solvent evaporation method. Factorial design was used to statistically optimize the formulation parameters. The particles were evaluated for entrapment efficiency, particle size, scanning electron microscopy (SEM), fourier transform infrared spectroscopy (FTIR) and differential scanning calorimetry (DSC). The drug release from the microsponge loaded gel was studied using Franz diffusion cell. Skin irritation of the developed formulation was studied on Albino wistar rats.

Results

The drug release study indicated that topical application of optimized gel enhanced the drug residence time in skin and therapeutic drug concentration was maintained up to 8 hrs. The developed gel formulation was effective against acne causing bacteria [3].

Discussion and conclusion

This study involved development of sustained release microsponge based gel for acne. The microsponge system was optimized by appropriate variation of parameters like drug-polymer ratio, volume of solvent (dichloromethane), stirring time and stirring speed. The microsponges were incorporated into a suitable gel base. It exhibited controlled release of azelaic acid and adapalene as compared to the marketed formulation. Skin irritation study indicated the microsponges to be non-irritant. Antibacterial study showed antibacterial activity of azelaic acid being retained on encapsulation. The developed microsponges proved to be a suitable sustained release topical delivery system of adapalene and azelaic acid for acne better than the marketed conventional delivery systems.

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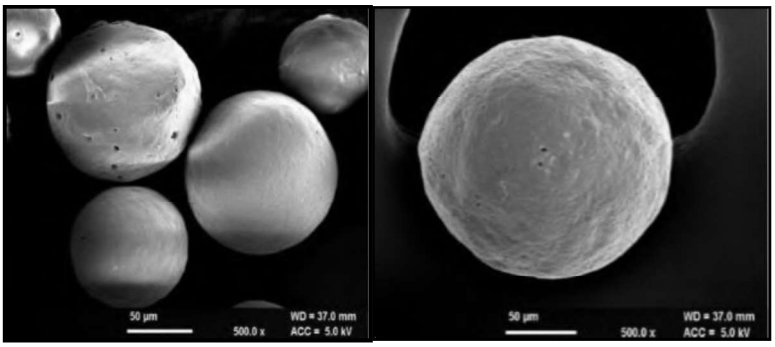

Fig. 1 SEM of optimized batch of microsponge

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Session 3, **O 4.**

Fluorescent polymeric nanocarriers of low cytotoxicity for two-photon bioimaging

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Background

With their strong photoluminescence, narrow emission peaks [1], high quantum yields [2] and significant two photon absorption (TPA) cross sections [3], colloidal semiconductor nanoplatelets (NPLs) are promising candidates for bioimaging and photodynamic therapies. However, bioapplications are limited due to their toxicity [4] and hydrophobicity which leads to aggregation of NPLs in biological liquids. Here, we present our method of preparation polymeric nanocarriers (NCs) in order to introduce hydrophilicity and lower toxicity of the inside-loaded semiconductor NPLs which does not change their optical properties.

Material and Methods

Polymeric NCs were prepared by vaporization of 5.5 ML CdSe NPLs dispersed in chloroform mixed with Pluronic 123 dichlorometane solution. The resulting concentrate was stirred overnight with distilled water.

Optical properties of the NPLs-loaded NCs, i.e. optical density and photoluminescence, was defined using spectroscopic methods. TPA cross section was calculated using two-photon excited emission.

Cytotoxicity of as-prepared material was determined using MTT assay on human gingival fibroblasts normal cell line and human ovarian cancer cell line.

Results

We obtained water-soluble spherical NPLs-loaded NCs of about 150 nm diameter with no significant change in photoluminescence signal or TPA cross section (up to 108 GM order of magnitude per one carrier) in wide range of wavelengths (670-1250 nm) in comparison to non-encapsulated NPLs. Our method provides cells viability up to 95%.

Discussion and conclusions

Preserved photoluminescence narrowness and high intensity as well as uniquely high

TPA cross section in the first biological transmission window [5] provide excellent optical properties for potential bioimaging application. High hydrophilicity and significantly lowered toxicity allow to use the material in any biological environment while extended size of the NPLs-loaded NCs is a step towards selective take-up by mutated cells.

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Session 3, **O 5.**

Mixture of MMP-2, MLCK and NOS inhibitors induce cardioprotection against myocardial ischemia/reperfusion injury

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Background

After myocardial infarction (MI), a large volume of mechanical function of myocardium is lost. It arises from heart injury due to generation of reactive oxygen species (ROS) during ischemia and postischemic organ reperfusion [1].

Nitric oxide (NO), is synthesized from L-arginine through a complex oxidation reaction catalyzed by NO synthase (NOS) and plays an important role in cardiovascular homeostasis. The levels and bioactivity of NO are regulated by eNOS (endothelial NOS), nNOS (neuronal NOS) and iNOS (inducible NOS) as well as endogenous NOS inhibitors such as asymmetric dimethylarginine (ADMA) [2]. During ischemia/reperfusion (I/R) a large amount of inducible nitric oxide synthase is produced with subsequent increase of ADMA [3]. ADMA disrupt nitric oxide signalling in endothelium by switch of the enzymatic activity from NO to ROS production. In consequence a toxic peroxynitrite (ONOO-) is formed that activates matrix metalloproteinase 2 (MMP-2). MMP-2 mediates degradation of contractile proteins and nitrates/nitrosylates of myosin light chain 1 (MLC1) and troponin I contributes to myocardial IR injury.

The aim of this study was to verify if co-administration of subthreshold doses of doxycycline (MMP-2 inhibitor), L-NAME (non-selective inhibitor of NOS) and ML-7 (inhibitor of MLC phosphorylation by MLCK – myosin light chain kinase) regulates of NOS-ADMA-NO pathway leading to cardioprotection.

Material and Methods

Cardioprotective effect of the drug cocktail was tested on isolated rat hearts by Langendorf method. Hearts extracted from anesthetized male Wistar rats (300-350 g) were perfused with Krebs-Henseleit buffer: after 25 min of aerobic stabilization, hearts were subjected no-flow ischemia (20 min)

in the presence or absence of inhibitors mixture (Doxy (1.0 μM), ML-7 (0.5 μM) and L-NAME (2 μM)) followed by 30 min of aerobic reperfusion. Next to hemodynamic parameters (coronary flow, heart rate, left ventricular developed pressure) biochemical markers of I/R injury were measured in a heart tissue and coronary effluents.

Results

Mixture of Doxy (1.0 μM), ML-7 (0.5 μM) and L-NAME (2 μM) increased heart function at 85% of aerobic control. The co-administration of subthreshold doses of inhibitors led to reduction of iNOS ($p<0.001$) and ADMA levels to the level approximate to aerobic control ($p<0.002$) and in turn increase in NO content to the level close to the aerobic control ($p<0.003$). Additionally, the positive correlation between iNOS and ADMA was found ($r=0.88$, $p=0,004$). Level of both iNOS and ADMA negatively correlated with NO content ($r= -0.83$, $p=0,009$ and $r= -0.96$, $p=0,001$ respectively).

The activity of MMP-2 in cardiac tissue of rats subjected to I/R was significantly higher compared to aerobic controls. Co-administration of subthreshold doses of inhibitors led to normalization of MMP-2 activity to the level of aerobic control ($p<0,005$). There was a positive correlation between MMP-2 and iNOS ($r=0.81$, $p=0,008$) as well as MMP-2 and ADMA ($r=0.78$, $p=0,02$).

Discussion and conclusions

Thanks to synergistic effect of drugs, the multidrug therapy with the subthreshold doses allows to address a few pathways of I/R injury simultaneously and to achieve protection of cardiac function during I/R. This study confirmed that co-administration of subthreshold doses of Doxy, ML-7 and L-NAME serves cardioprotective. Additionally, this study provided an important insight into understanding the interaction of iNOS, eNOS and ADMA, which is crucial for development the therapy beneficial for patients after myocardial infarction.

This work was supported in part by the National Science Centre, grant number UMO-2016/23/B/NZ3/03151

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Session 3, **O 6.**

Personalization of pancreatic cancer treatment- electroporation, electrochemotherapy and calcium electroporation, pilot study

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Background

Pancreatic cancer has a very poor prognosis. Despite of the development of cancer biology knowledge, radiology, cancer treatment all over the world this cancer has the same high mortality to morbidity index from years.

The purpose of the study was to investigate safety of electroporation, electrochemotherapy and calcium electroporation in pancreatic cancer treatment. Qualification of the patients ("therapeutic moment"), safety and complications after procedures were examined.

Material and Methods

The group of 12 patients in this pilot study was operated in 2-nd General and Oncological Surgery, Medical University of Wrocław, Poland. Main inclusion criteria were pancreatic cancer in all stages and recurrent disease. Patient underwent electroporation (IRE – irreversible electroporation) or electrochemotherapy (ECT – electrochemotherapy) with intravenosus admission of cisplatin or electroporation with calcium intratumoral administration.

There was 1 patient with resectable pancreatic cancer, 8 with non- resectable, locally advanced pancreatic cancer, 1 with metastatic disease and 2 with recurrence. In 4 patients only electroporation was administrated, 4 were with intratumoral calcium ions administration, 2 with intravenosus cisplatin and 2 with both calcium and cisplatinum administration.

Results

The surgical and anaesthesiological procedure was safe. There were 2 post- operative complications: bile infection connected to the surgical by- pass procedure and mild pancreatitis connected to the IRE procedure with calcium ions administration. There was also one 30-th day death because of circular insufficiency and fragile syndrome according to patient with oligometastatic disease.

Discussion and conclusions

Electroporation procedure is safe for enchancement of the surgical procedure and for patients with non-resectable locally advanced pancreatic cancer (LAPC). Patients with metastatic disease require caution in qualification because of fragile syndrome.

Intravenosus cisplatin administration is safe and calcium ions intratumoral administration requires further investigation.

The best "therapeutic moment" for each patient should be considered separately with interdisciplinary group of study.

These are only pilot studies and the results are only observation rather than guidelines. Also the sufficiency of these methods to the overall survival (OS), disease free survival (DFS) and progression free survival (PFS) needs to be continued, because of too short time of observations.

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Session 4, **O 1.**

Thiazole based derivatives as sirtuins inhibitors. Molecular docking study

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Background

Sirtuin subtypes have been identified in mammalian cells which are responsible for the regulation of cellular metabolism, transcription and differentiation processes. Through a large number of their substrates sirtuins are able to determine the biological life expectancy, including mitosis, apoptosis, DNA repair and metabolism [1]. They are assigned to the III class of histone deacetylases, but are able to catalyze the deacylation of various non-histone reagents. Therefore, human sirtuins represent therapeutic target for the treatment of metabolic dysfunctions, neurological disorders, age related conditions and cancer [2]. To date many different potential inhibitors of sirtuins have been described [3]. Unfortunately, most of them show average rather small potency and low selectivity. Therefore, it is extremely desirable to develop a new more potent and highly selective compounds. It is widely known that drug discovery is time consuming and expensive process. The application of computational techniques in design and optimization of novel compounds allows to omit the traditional procedure and reduce time and cost of drug development process.

Material and Methods

Within the presented project, several computational methods were used in order to design the new compounds based on thiazole scaffold. We applied the structure-based drug design approach where the information about the three-dimensional structure of molecular target is important. The geometries of analyzed derivatives were optimized based on density level theory. Molecular docking simulation was performed in order to obtain the binding mode of proposed inhibitors towards sirtuin-2. Additionally, the qualitative and quantitative analysis of intermolecular interactions was performed.

Results

The results obtained during molecular docking revealed that all investigated compounds are able to bind to the active center of sirtuin. The most stable complex is characterized by the lowest binding free energy (-32.6 kJ/mol) and also the lowest inhibition constant (1.94 μM). As can be observed in this case the aromatic rings of the ligand were exposed to hydrophobic amino acid residues, mainly Phe96, Leu103, Phe119, Ile169, Phe190, Val233 and Leu 239 (See Fig. 1). There is also possibility of H-bonding interactions involving Arg97 and Val233 residues.

Discussion and conclusions

The analysis of the issues presented here is an important contribution to the problem of design of selective inhibitors of sirtuins. We characterized in details the binding mode of thiazole derivatives in the binding site of sirtuin-2 what is a further step towards better understanding the molecular recognition process and mechanism of inhibition. According to the previous studies the proposed compounds might be involved in interactions inside the induced hydrophobic pocket similar to myristoylated-lysine substrates [4]. All analyzed complexes are stabilized mainly by hydrophobic and van der Waals forces. Moreover, the inhibition

constant allowed to predict the ability of proposed ligand to inhibition of protein and might be correlated to the half-maximal inhibitory concentration. Finally, the proposed derivatives may be used as lead compounds for drug development to further studies.

Acknowledgments
Authors gratefully acknowledge the allotment of the CPU time in Wroclaw Center of Networking and Super- computing (WCSS).

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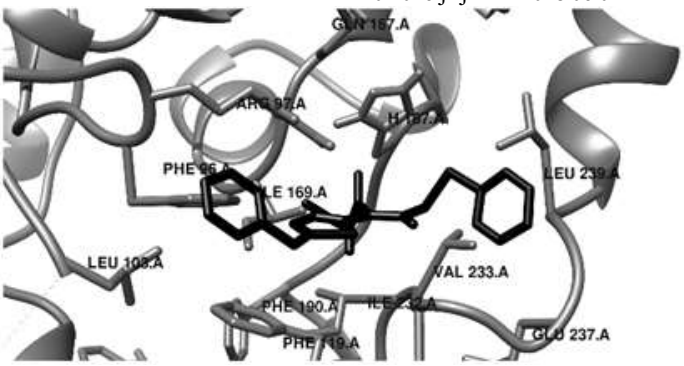

Figure 1. The binding mode of the most potent compound

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Session 4, **O 2.**

Fighting cancer with gravity

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Background
Numerous studies have reported that gravity alteration has remarkable influence on growth and biological processes of human cells. Most studies have tended to focus on the impact of altered gravity on human cells; however, issues linked to malignant cells have not been dealt with in depth. Therefore, gravity-related experiments have become an promising method to improve our knowledge in the field of cancer biology and may be useful to detect interesting implications for future cancer treatment. Taking this concept further we studied the effect of simulated gravity (sμg) on human cancer cells using Random Positioning Machine (RPM).

Material and Methods
In an attempt to determine whether altered gravity might be one of the factors modulating multidrug resistance (MDR) in cancer cells we used well defined commercial

human ovarian cancer cell line SKOV-3 resistant to cisplatin and doxorubicin. The cells were seeded on T25 cell culture flasks fully filled with growth medium (without the presence of air bubbles) and exposed to simulated microgravity for 2h in the presence of cisplatin as a model of cytostatic drug administered directly before the experiment. After centrifugation, the cells were detached and seeded on 6-well and 96-well plates for 24 and 72 hours to perform cytotoxicity, proliferation, cell death and cell cycle analyses. Additionally, the cells were cultured on coverslips and fixed directly after the centrifugation to evaluate cell morphology using 3D Cell Explorer (Nanolive), confocal and scanning electron microscope.

Results

Our studies revealed that SKOV-3 cells are susceptible to simulated microgravity which affects cell morphology and drug efficiency. We observed altered cell shape, presence of membrane blebbing, lack of lamellipodia and intracellular rearrangement of cytoskeletal fibres (actin, β tubulin and zyxin) even when the cells were cultured on RPM for 2 hours (Fig. 1). Cytotoxicity and cell death assays showed increased percentage of apoptotic cells after centrifugation on RPM in the presence of cisplatin in comparison to control, not centrifuged cells. Additionally, clonogenic and cell cycle assays revealed decreased percentage of proliferative cells and G1/G0 arrest.

Conclusions

We believe that gravitational stress may affect cell pathways involved in multidrug resistance phenomena, especially associated with cell membrane and cytoskeleton, resulting in higher sensitivity of cancer cells to chemotherapeutics. The investigation and clarification of these phenomena may constitute initial step toward enhancing our understanding of the relationship between cellular resistance to chemotherapy and the response to various gravitational stimuli. In our view this experiment constitutes an excellent initial step toward enhancing our understanding of the relationship between cellular resistance to chemotherapy and the response to gravity alteration.

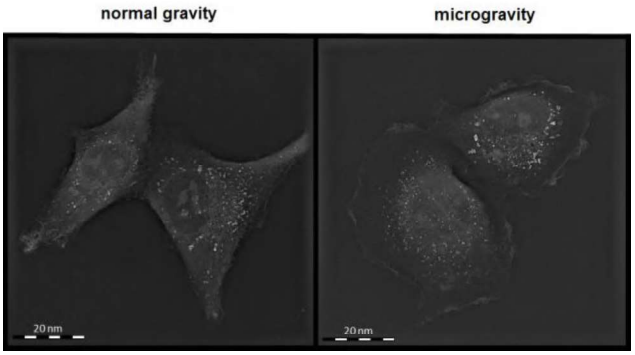

Figure 1. SKOV-3 cells morphology evaluated using 3D Cell Explorer microscope after culturing for 2h on RPM.

Session 4, **O 3.**

Combination of EP and PDT in melanoma treatment

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Background

Curcumin is widely known for its high potency as an anticancer drug [1]. Due to its hydrophobic properties, it seems to be especially effective towards cutaneous and subcutaneous tumors. Nowadays more and more effort is being devoted to enhance cytostatic properties of the drug with the use of novel therapies, such as photodynamic therapy (PDT) and electrochemotherapy (ECT). Both of them show promising effects when applied alone, but recent data suggest, that their combination can be beneficial [2].

In this project, the authors propose a protocol for effective combination of PDT and ECT, validated by a set of experiments.

Material and Methods

The experiments have been performed on melanotic (A375) and amelanotic (C32) cell lines, while fibroblasts have been used as a model of non-cancerous cells. To study the PDT and ECT protocols, we analysed the effects of irradiation and of high electric fields on curcumin using mass spectrometry methods. Immunofluorescence staining studies as well as viability tests were performed on all cell lines. The interaction of curcumin and its derivatives with model cell membranes, namely lipid bilayers, was studied using molecular dynamics simulations.

Results

Our analyses show that during PDT, curcumin undergoes decomposition to more potent and smaller compounds, such as vanillin and ferulic acid. In ECT on the other hand, curcumin loses sequentially its methoxy groups. Due to its rather hydrophobic nature, curcumin first partitions within the lipid membranes (cells envelop). With time, it changes its localization to intracellular membranes.

Overall concerning the effectiveness of using curcumin as anticancer agent, the preincubation with curcumin has led to much worse results.

Discussion and conclusions

Two hypotheses can explain the obtained results: (1) either irradiation of the photosensitizer disrupts the membranes in which it localizes, leading to extensive damage; (2) or inside the cells, curcumin metabolism being rapidly metabolized, the effectiveness of PDT is drastically reduced.

At any rate, the data we have gathered show that the most effective way of combining both therapies is to electroporate simultaneously after addition of the drug and irradiate afterwards.

Further studies are now required in order to test whether these *in vitro* protocols to effective successful cancer therapy.

Acknowledgments:

This research was supported financially by the Subsidy Funds of Department of Molecular and Cellular Biology SUB.D260.20.009

The authors would like to thank dr Hanna Czapor-Irzabek (Elementary Analysis and Structural Studies, Wrocław Medical University, Poland) for the assistance in mass spectrometry analyses and expert support.

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Session 4, **O 4.**

Pulsed Current Evaluation for Prediction of Tumor Permeabilization Rate

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Background

Electroporation is a phenomenon of increased biological cell membrane permeability facilitated by pulsed electric fields (PEF) [1]. One of the most established biomedical applications of electroporation is electrochemotherapy, when chemotherapeutic agents are delivered to the tumour by increase of cellular membrane permeability [2, 3]. However, during the clinical procedures, usually there is no feedback on the efficacy of the treatment and the success rate of the procedure is unknown until several days post-treatment. In this work we tested the feasibility of pulsed current measurements to serve as a real-time indicator of successful tumour permeabilization by electric fields.

Material and Methods

BALB/c mice were bred and housed in mouse facility of State Research Institute Centre for Innovative Medicine, Vilnius, Lithuania. 1×10^6 of SP2/0 myeloma cells in phosphate-buffered saline (PBS) were inoculated under the skin on the back of the 6–8 weeks old mice. The tumors were allowed to establish and grown until they reached ~150-500 mm^3 and were ready to treat. 12 mg/kg of doxorubicin (Ebewe pharma, Austria) was injected intraperitoneal 15-20 minutes prior to the treatment. Up to 3 kV, 100 ns – 1 ms square wave high voltage and high frequency (up to 1 MHz) pulse generator was used for electroporation. Two electroporation protocols were employed: 1) 1.4 kV/cm x 100 µs x 8 pulses and 2) 3.5 kV/cm x 800 ns x 1000 pulses. Needle electrodes with a gap of 5 mm were used for pulse delivery.

For *in vitro* experiments, commercially available electroporation cuvette with 1 mm gap aluminum electrodes (Biorad, Hercules, USA) was used and the parametric cell permeabilization curve was acquired using Propidium Iodide (PI, 45 µM) (Sigma-

Aldrich, Germany) and flow cytometry (Amnis, Seattle, USA).

All experimental protocols were approved by the Lithuanian State Food and Veterinary Service (approval no. 02-24) and carried out in accordance with the the Guide for the Care and Use of Laboratory Animals.

Results

During microsecond pulse procedure the pulses were delivered with a 30 s delay to prevent any influence of Joule heating. A maximum 9% increase of current was detected between the first and the last pulse, however, the increase of current was statistically significant only between the first and (3-8 pulses) ($P<0.05$, $n = 3$). To summarize, the current was increasing during the first 3 pulses, followed by a saturation when higher number of pulses was applied.

Similar tendency was observed during nanosecond pulsing procedure. A distinguishable increase of the current between the first and the second pulsing bursts was detected. The variation of current for all the other pulse sequences (200–800 pulses) was not statistically significant.

In vitro data in the microsecond pulse range indicated that the permeabilization rate of the cells is saturated (>95%) after 4th pulse, which is in agreement with current increase tendency. In case of nanosecond protocols, the permeabilization rate is saturated after the first 100 pulses, which is also in agreement with *in vivo* current measurements.

Both protocols triggered a statistically significant tumor response to electrochemotherapy.

Conclusion

It was shown that the changes in current can serve as an indicator of electroporation. The result was confirmed both *in vitro* and *in vivo*, however, the methodology is limited to non-thermal PEF treatments.

Acknowledgement

The research was funded by Research Council of Lithuania, Grant Nr. S-MIP-19-22.

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Session 4, **O 5.**

A multifunctional nanoprobe based on polymeric nanocapsules loaded with quantum dots and lanthanide doped nanocrystals

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Colloidal quantum dots (QDs) exhibit several numbers of unique size-dependent optical properties, e.g. narrow band-gap emission and large absorption cross-sections. Moreover, they are interesting candidates for bioimaging because of large one- and two-photon absorption cross-sections. On the other hand, inorganic upconverting nanocrystals can be used for designing new bio-medical markers due to good optical stability, low toxicity, and high signal-to-noise ratio of the up-conversion emission. They are excited by near-infrared (NIR) radiation, which can convert into visible emission. The main advantages of NIR excited emission is high penetration depth through many materials including biological tissues and the possibility of applying relatively cheap and easily accessible continuous wave (CW) laser diodes.

One primary problem with bioapplication of QDs and upconverting nanocrystals is their transfer to an aqueous solution. To overcome this problem, some approaches have been made including using biomolecules, such as proteins, as the capping agents for quantum dots [1] as well as encapsulation of the nanocrystals within a polymer[2].

For the purpose of designing multifunctional nanoprobe dispersed in water, hydrophilization of colloidal CdSe QDs and $NaYF_4$:Yb,Er nanocrystals into hydrid structures was employed using encapsulation process of nanocomponents.

In order to obtain nanocapsules with the most effective properties, we prepared series of samples with different nanoparticles concentrations. Firstly, we studied optical properties of nanocapsules dispersed in water. Next, we investigated individual nanocapsules using scanning confocal fluorescence microscope, equipped with piezo-electrically controlled sample holder and high NA oil-immersion objective. The structure and morphology of samples were characterized with X-ray scattering technique and transmission electron microscope measurements.

We conclude that encapsulation method can combine the features of two kinds of nanocrystals into a single architecture and it is possible to obtain dual emission from two kind of nanocrystals (CdSe QDs and $NaYF_4$:Yb,Er at the same time). The determined luminescence properties indicate that the $NaYF_4$:Yb,Er/CdSe QDs assemblies are efficient imaging agent dispersible in aqueous solution.

Acknowledgements:
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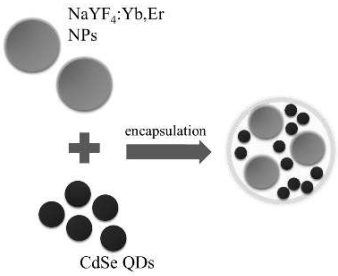

Fig.1. Schematic graph of encapsulation processes of nanocomponents

Session 4, **O 6.**

Light-induced in situ TEM microscopy revealing ultrastructural interactions in antimicrobial photodynamic therapy

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Background

The team's main research topic is the development of antimicrobial photodynamic therapy (aPDT) methods [1] and the development of appropriate lighting sources for its purposes [2]. The project described below aimed at understanding the ultrastructural mechanisms that lead to the therapeutic effect of aPDT.

Material and Methods

The basis for modification was Hitachi H-800 microscope, with a tungsten thermal emission and high voltage of 200kV. To make the liquid cell sample containing bacteria and photosensitizer, the double 15 nm amorphous carbon on the copper grids were used. The in situ illuminator mounted inside the TEM column contained 660 nm LED emitter and applicable light pipe. The light intensity was calibrated to achieve 0.5 mW/cm^2 on the sample. The microorganism used for photodynamic therapy imaging and survival tests was *Staphylococcus aureus*. The photosensitizer used during in situ aPDT was a solution of methylene blue in PBS at a concentration of 250 µg/mL.

Results

The method used allowed observation of bacteria surrounded by a photosensitizer and observations in a native, hydrated state (fig. 1). Preliminary observations shown the morphological changes in the cell wall caused by the generation of singlet oxygen during PDT. Dissection and disintegration of the bacterial outer shell and peptidoglycan layer allowed photosensitizer infiltration near the cell membrane. This lead to cell lysis and bacterial death.

Discussion and conclusions

The performed modification of the TEM device shows that a certain group of dynamical in situ biological observations could be carried out in transmission emission microscopes. this means that the sample does not need to be typically fixed, and can be influenced during observation. This requires a suitably short electron beam interaction but offers the opportunity to observe dynamic phenomena on a true nanometric scale.

The authors gratefully acknowledge funding from the National Science Centre (PL) under "Miniatura" grant number 2019/03/X/NZ3/02100

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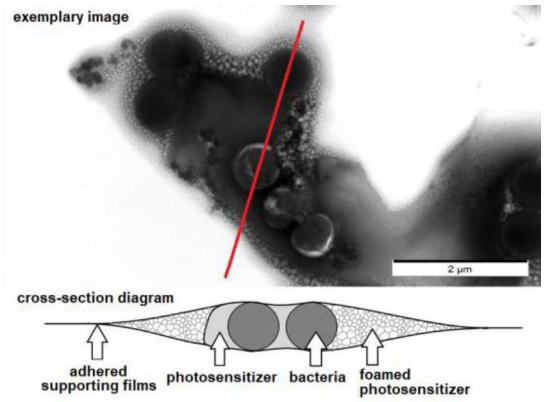

Figure 1. The exemplary TEM image of a prepared liquid cell and cross-section diagram

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Session 6, **O 1.**

Life support system in a remote Mars colony

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Background

The concept of the "Twardowsky" Mars colony for 1000 people was granted second place in The Mars Colony Prize 2019. Further research on the colony's life support systems led to publishing a paper [1] which deepens and thoroughly analyses proposed solutions. Mars harsh conditions lead causes multiple problems to encounter in terms of life support. The reduced gravity, lack of breathable atmosphere and insufficient recourses to name a few. Based on the cutting-edge technology and space research there are numerous solutions to those problems. In this study it is aimed to introduce a closed-loop system which could potentially sustain human presence on the Red Planet. Four depended systems were introduced and described: atmosphere system, solid waste system, water and wastewater system and biomass system.

Material and Methods

The conducted research was based on the up-to-date information regarding human organism behavior in extra-terrestrial conditions. Additionally, numerous analysis and simulations were conducted in scope of the waste management and daily dietary demand. A variety of organic waste processing methods were investigated with respect to supporting 1000 inhabitants in environment of the planet Mars.

Results

As a result of research, life support systems for remote mars colony has been proposed and published [1].

An atmosphere, biomass, solid waste, water and wastewater systems was presented, and their functionality analyzed in terms of life support in Martian conditions for 1000 people.

Processing methods and specific solutions were described. Mathematical simulations of the bioreactor flow and waste management were presented and calculated with redundancy.

Discussion and conclusions

The introduced concept gave a proof of technological feasibility and showcased a problem of subsystems implementation as a unity. Moreover, the emphasis (followed by calculations) was put closed loop of resources and efficiency of the proposed processes regarding life sustainment.

There are certain research problems regarding the described systems that should be examined in the foreseeable future.

- atmosphere subsystem: reducing the weight, efficiency improvement, reduction of energy demand
- water subsystem: inoculation of biological reactors, the effect of reduced gravity on the aeration system and the collection of excessive sludge, recovery of elements from brine from physicochemical devices
- solid waste subsystem: significant improving the efficiency, recovering of valuable chemical compounds and development of technology to process them
- biomass subsystem: clogging of aeroponics, precipitation in each system, collecting harvests by the crew.

References:

List your references here, and use the example below:

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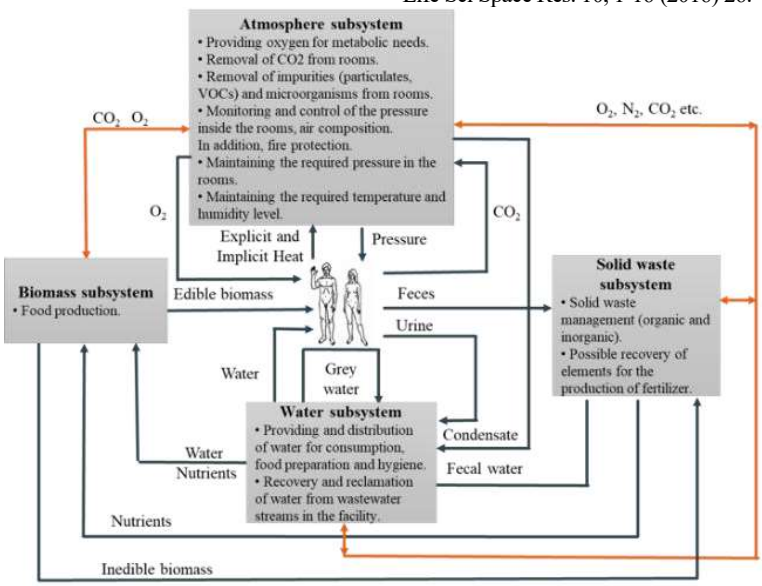

Fig.1 Scheme of life support systems dependencies

Session 6, **O 2.**

Changes in nuclear proteome associated with lamin in the *Drosophila melanogaster* model system after heat induction

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Background

One of the best examined extracellular stressors is heat-shock induction. Cells in response to increased temperature have developed an evolutionarily conserved process- heat shock response (HSR). During HSR the heat shock transcription factor (HSF) binds to the promoters of hsp (heat shock proteins) genes resulting in activation of heat-inducible genes and a global down-regulation of transcription. Moreover, it has been found that after heat shock induction the decondensation of chromatin occurs [1].

Changes in interaction between chromatin and protein after heat shock were also observed with major karyoskeletal proteins involved in chromatin organization – lamin. It belongs to V-type intermediate filaments exerting structural and regulatory functions in the cell nucleus [2]. Our hypothesis is that lamins, together with topoisomerase II (Top2 is an enzyme required for DNA regulations) may play a key role in chromatin remodeling during HSR.

For our studies, we chose *Drosophila melanogaster* as a model system due to the presence of only two lamin genes – B-type (lam Dm) and A-type (lam C) and a single isoform of HSF, which makes it a definitely simpler model than vertebrates. In this study, we focused on investigating differences between normal and heat shock condition with regard to changes in protein complexes associated with lamin Dm together with post-translational modifycations which may be crucial in processes occurred during HSR.

Material and Methods

All experiments were performed on *D. melanogaster* embryonic cell line – Kc. Cells were maintained in suspension culture (in Schneider`s Drosophila Medium from Gibco with 10% FBS and 1% antibiotics) at 23°C as normal conditions. To induce the heat shock cells were incubated at 37°C for 1 h before further experiments. To identify proteins interacting with lamin 1% PFA cross-linking (10 min, RT) followed by co-immunoprecipitation (co-IP) under denaturing conditions (based on the protocol from ThermoFisher dedicated to Pierce Protein A/G Magnetic Beads). Samples after co-IP were next digest by FASP method, tryptic peptides were analyzed by tandem mass spectrometry analysis (LC-MS/MS). MS/MS data were processed using the Mascot searching engine (UniProt *Drosophila* database combined with The common Repository of Adventitious Proteins, cRAP).

Results

We aimed to confirm the interaction between lamin Dm and topoisomerase II in both, normal and heat shock conditions. We observed extreme change in the number of proteins identified in MS after heat shock (almost 70 more interactors identified in comparison to control). After the classification of identified proteins, we observed changes in clusters in both groups based on protein functions. In HS samples we observed an increased number of proteins involved in DNA/RNA binding. Based on the quantitative analysis we showed about 30% decreased of lamC identifiers (the best-

known interactor of lamDm, q-value= 0,03), 30% increase of Top2 identification after hs (but the result is ns).

Discussion and conclusions

Previous experiments suggest that lamin and topoisomerase II are involved in the regulation of transcription during heat shock induction and moreover they interact directly with chromatin. We showed the interaction between them and along with other protein identifications from co-IP experiments its confirm us in this belief. To determine whether the interaction is direct or indirect (through chromatin) further experiments have to be performed (co-IP with nucleic acid digestion). Changes in lamin- interacting proteome may be the result of the re-localization of lamin Dm after induction of heat shock or might be the effect of different phosphorylation rates in both conditions. Observed protein pattern of interactors with lamin Dm after heat shock induction leads us to conclude that lamins may play a role in the epigenetic shutdown of transcription after heat shock-induced together with other components of a protein complex involved.

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Session 6, **O 3.**

Antiviral activity of extract of Ginkgo biloba (EGb) and its phytochemical constituents against herpesviruses HHV-1 and HHV-2

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Background

Human alphaherpesvirus 1 (HHV-1) and *Human alphaherpesvirus 2* (HHV-2) belong to the most common worldwide infections of humans, producing a lifelong infection. Several factors, such as immune impairment, UV or stress, lead to virus reactivation in the place of the initial infection (oral cavity, lips or genital organs), causing pain and skin ailments [1]. Despite the availability of several anti-herpesviral agents, it should be emphasized that the need for new inhibitors is highly encouraged due to the increasing resistant viral strains as well as complications linked with periods of recurring viral replication and reactivation of latent herpes infection [2]. The most promising are preparations of a natural origin, such as extract of *Ginkgo biloba* (EGb) [3], as an alternative to commercially available synthetic preparations. We evaluated the antiviral activity of EGb and its phytochemical constituents: flavonoids and terpenes against HHV-1 and HHV-2.

Material and Methods

Standardized dry extract from *G. biloba* leaves, mix of flavonoids and mix of terpene lactones as well as single flavonoids from *G. biloba*: isorhamnetin, kaempferol, and quercetin were investigated. Inhibition of HHV-1 and HHV-2 replication was examined by early viral entry assay (inactivation assay). Serial concentrations of EGb and its phytochemical components were

incubated with HHV-1 or HHV-2 for 0,5 h (Short-Term), 1h and 2h (Long-Term). Viral titer was expressed with reference to the $TCID_{50}$ (tissue culture infectious dose) value, based on the cytopathic effects (CPE) caused by the virus in approximately 50% of infected cells. An appropriate Gompertz growth model and exponential model were fitted to estimate the concentration-dependent decrease in virus titer after treatment with EGb and its phytochemical components.

Results

Pretreatment of the herpesviruses with EGb, mix of flavonoids, mix of terpene lactones and flavonoids from *G. biloba*: isorhamnetin, kaempferol, and quercetin prior to infection of cells were studied. EGb produced a remarkable anti-HHV-1 and anti-HHV-2 activity. The extract affected the viruses before adsorption to the cell surface at non-cytotoxic concentrations, what is an important benefit of this extract. Even by 4 log $TCID_{50}$ reduction of both viruses titer with EGb was observed, which means a 99.99% decrease in infectivity. Flavonoids from EGb, especially isorhamnetin, are responsible for the antiviral activity of the extract. Such activity was absent in quercetin and kaempferol. However, EGb showed the most potent antiviral potency compared to isorhamnetin. We have investigated also an antiviral activity of EGb against other viruses belonging to different taxonomic groups such as *Human adenovirus 5 (*HAdV-5), *Vesicular stomatitis virus (*VSV) and *Enteric cytopathogenic bovine orphan virus (*ECBO). EGb, however, did not express antiviral activity against any of these viruses. A strong antiviral activity of EGb was observed only for herpesviruses.

Discussion and conclusions

Standardized EGb shows high anti-HHV-1 and anti-HHV-2 activity in non-toxic concentrations and significantly reduces the infectivity of both pathogens. Most likely, EGb could augment current therapies for herpes labialis and genital herpes, especially in the treatment of skin ailments during recurrent infections [4]. A combination of antiviral agents with different molecular targets, including EGb, has the potential to keep HHV-1 and HHV-2 replication to a minimum.

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Session 6, **O 4.**

Trimethylammonium 1-mercapto-1-carbadodecarnorate (TMA) – pharmaceutical precursor in BNCT method

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Background

Statistical data clearly indicate an increase in the incidence of tumours, every year. Probably, that is a result of the changing lifestyles and the progressive globalization. There are two factors crucial for the successful treatment of the disease, these are the early detectability and the specificity of the applied therapy. We need new compounds for the effective detection and elimination of tumours. Boron Neutron Capture Therapy (BNCT) [1, 2] selectively targeted the tumour cells can be an effective solution to this problem. The therapy belongs to the so-called bi-modal therapies: (1) a pharmaceutical containing molecules that preferentially penetrate cancer cells and are not absorbed (or little absorbed) by healthy cells are administered to the patient. The molecules are labelled with boron stable isotope ^{10}B; (2) the patient's body is irradiated with epithermal neutrons of some specific energy. Thermal neutrons are preferentially captured by boron nuclei. Having a neutron captured, stable ^{10}B nucleus transforms into a meta-stable ^{11}B nucleus(1), which almost instantly decays along one of the following paths (1).

Both particulate reaction products (^{7}Li and α-particle) transfer their energy into the surrounding tumour tissue destroying her. Therefore, the high accumulation and selective delivery of ^{10}B into the tumour tissue are the most important requirements to achieve efficient BNCT therapy. Three important parameters should be considered in the development of boron carriers: (1) the boron concentrations in the tumour should be in the range of 20-35 mg ^{10}B per g; (2) the tumour/normal tissue ratio should be greater than 3-5; and (3) the toxicity should be sufficiently low [1].

That the three principles above (1-3) be met, searching for new carriers of boron-10 is both necessary and important [1, 3]. The aim of this project is to characterize a new carrier for clusters of boron-10: trimethylammonium 1-mercapto-1-carbadodecarnorate (TMA). The selected chemical compound has an analogous structure for clinically used sodium mercaptododecaborate (BSH). TMA has a -SH group that enables coupling reactions with neurotransmitter proteins (targeted therapy).

The aliphatic chain increases lipophilicity, facilitates crossing the blood-brain barrier (brain cancer therapy).

Material and Methods

In this work, there were used a number of chemical and biological methods to study TMA and BSH compounds, including durability in serum, lipophilicity, receptor affinities, toxicity, IC50 and apoptotic pathway. Two cells cultures were used: colon cancer cells HCT116 and healthy colon cells CCD841, and two boron compounds: mercaptododecaborate dianion, trimethylammonium 1-hydroxy-1-carbadodecaborate were used in this work [4].

Results

Due to their structure, TMA and BSH are easily coupled with proteins. Additionally, by dint of showing poor EPR signal, TMA is detectable in biological structures. Both compounds crossed the cell membrane and located in the cytoplasm. Survivability of the cells correlated with cytotoxicity of the compounds tested. The apoptosis pathway is contingent on the concentration of the compound tested, not on the incubation period.

Discussion and conclusions

It is highly durable in solutions of diverse pH and serum. Consequently, as a pharmaceutical, it could be administered orally. Moreover, it is stable in all conditions tested and has proper lipophilicity. It does not exclude the ability to cross the blood-brain barrier. The properties pointed out to create an opportunity to enhance bioaccessibility

and extending of the half-life of the compound.

The project is financed from National Science Centre (R. No. 2018/02/X/NZ7/ 03011).

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$$^{10}_{5}B + {}^{1}_{0}n \rightarrow [{}^{11}_{5}B] \begin{cases} \nearrow {}^{4}_{2}He + {}^{7}_{3}Li + 2.79\ MeV\ (6\%) \\ \searrow {}^{4}_{2}He + {}^{7}_{3}Li + \gamma 0.48\ MeV + 2.31\ MeV\ (94\%) \end{cases} \quad (1)$$

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Session 6, **O 5.**

The structure of Nucleobindin-2 is regulated by divalent metal cations

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Background

Nucleobindin-2 (Nucb2) is a multidomain protein, which possesses six domains: signal peptide, Ile/Leu-rich region, DNA-binding domain, two EF-hands domains, acidic rich region and leucine zipper motif [1]. The EF-hand domain is responsible for Ca^{2+} and Mg^{2+} binding [2]. Nucb2 exhibited a broad range of expression in central nervous system [3] and peripheral tissues [4], which suggests that Nucb2 is involved in variety of physiological processes. The aim of this study was to reveal the effect of Mg^{2+} and Zn^{2+} binding on the structure of recombinant Nucb2 from red junglefowl (*Gallus gallus*). Up to now CD spectroscopy and limited proteolysis were applied. The CD analysis showed that only Zn^{2+} binding resulted in alteration of the protein structure. This observation was consistent with limited proteolysis data. Only Zn^{2+} addition provided clear protection of Nucb2 against proteolytical degradation. Among divalent metal cations, Mg^{2+} and Zn^{2+} are the most abundant ones in the human body. The modulation of the protein structure by divalent metal cations may be significant for both, protein function and interactions.

Material and Methods

1. Purification: *Gallus gallus* Nucb2 was expressed in *E. coli* cells. Pure and non-tagged Nucb2 was obtained in four steps: immobilized ion metal affinity chromatography (IMAC), His-Tag removal by HRV3C protease, second IMAC and gel filtration. The purity of the protein was confirmed by SDS-PAGE and ESI-MS.

2. Circular dichroism (CD): The far-UV CD spectra were recorded with JASCO J-815 spectropolarimeter. The measurements were carried out for 10 μM Nucb2 in the absence and presence of Mg^{2+} and Zn^{2+} at 20°C from 300 nm to 195 nm in triplicate. The baseline spectra of the buffer were subtracted.

Secondary structure content was calculated using CDNN software [5].

3. Limited proteolysis: 10 μM ggNucb2 was digested with endopeptidase Glu-C (V8) (1:5000) in the absence (5 mM EDTA) and presence of 10 mM Mg^{2+} and 100 μM Zn^{2+}. The reaction was stopped at different time intervals and analysed by SDS-PAGE. The digestion was also performed in broad range of Mg^{2+} and Zn^{2+} concentration for 120 min.

Results

The far-UV CD spectra recorded in the absence and presence of Mg^{2+}/Zn^{2+} have two negative maxima at 208 and 222 nm, which are characteristic for α-helical structure [6]. However, the spectra deconvolution showed that Nucb2 has a significant amount of disordered regions too. The Mg^{2+} exhibited no significant effect on the secondary structure of Nucb2. However, Zn^{2+} increased the amount of α-helices and decreased the content of unordered structure. Limited proteolysis results showed that digestion patterns in the absence and presence of Mg^{2+} are similar, which is in agreement with CD spectra results. The V8 digestion resulted in generation of two fragments: of 45 kDa and 30 kDa. Interestingly, the presence of Zn^{2+} led to limitation of accessibility of cleavage site of Nucb2, which was caused probably by the conformational change of Nucb2 molecule. Presence of 100 μM Zn^{2+} led to accumulation of fragments of 45 kDa, which seemed to be proteolytically resistant. Low Zn^{2+} concentration (0-10 μM) has a minor effect on the susceptibility to V8 digestion (similar to Mg^{2+}).

Discussion and conclusions

In this paper, we utilized CD and limited proteolysis for initial characterisation of the effect of divalent metal cations on *Gallus gallus* Nucb2 and showed Zn^{2+} as the specific ligand for the protein. In particular, both secondary and tertiary structure of Nucb2 are modulated by Zn^{2+}. Interestingly, Mg^{2+} has not affected Nucb2 structure. The CD spectra showed that Nucb2 has a significant amount of α-helical structure and unordered regions. The addition of Zn^{2+} led to an increase of the α-helical structure and simultaneous decrease of the unordered regions. The molecule of Nucb2 in the presence of Zn^{2+} undergoes structural changes, which probably leads to attain a more compact structure. The limited proteolysis results also confirmed this hypothesis. Proteolysis occurres mainly at the unordered regions rather than α-helices [7]. The addition of 100 μM Zn^{2+} provided a protection of Nucb2 against V8 digestion. This Zn^{2+}-dependent conformational change of Nucb2 may have implication in the physiological role of this protein. However, the exact role of Nucb2-Zn^{2+} interaction required the further research.

Acknowledgement

The work was supported by the NCN Grant 02NO/0012/19.

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Session 6, **O 6.**

Stress affects the expression of 'major housekeeping' genes – is phosphorylation involved?

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This work was funded by the Opus 11 grant from the National Science Centre, Poland no. O-2016/21/B/NZ4/00541

Background

The process of transcription is one of the key adaptive mechanisms and needs to be strictly controlled in response to environmental factors and stimuli (as heat shock). Recently, interest in this topic has been growing among scientists, because it is still not known much about the mechanisms controlling it.

Heat shock is an invaluable model for studying mechanisms regulating gene expression and is well known and easy to control [1]. Many papers report that during stress, transcription is shut down globally while only a few loci are highly activated [2]. These active loci are connected with heat shock proteins (Hsp) family which functions as intra-cellular chaperones.

Lamins are evolutionarily conserved proteins classified as type V intermediate filaments, which are involved in the regulation of gene expression, chromatin organization, DNA replication and repair, signaling, developmental regulation, and nuclear positioning [3]. In order to play such a variety of functions, lamins interact with many different nuclear proteins, which are directly or indirectly responsible for a particular function. Lamins (the main component of the nuclear envelope) together with associated proteins built a complicated platform for the regulation of nuclear processes. It has been proved that the chromatin regions located near the nuclear envelope consist mainly of heterochromatin – transcriptionally inactive regions.

Our research suggests that lamins and associated/interacting proteins are significantly connected with transcription regulation. In this work, we focus on changes in gene expression profile in stress response. Our results also suggest that the phosphorylation status of HSF and lamins changes. Does the phosphorylation cause the transcription shut down or is it the result of it?

Material and Methods

Cell culture and heat shock treatment

All experiments were performed on *D. melanogaster* embryonic cell lines – Kc and S2. Cells were maintained in suspension culture (in Schneider`s Drosophila Medium from Gibco with 10% FBS and 1% antibiotic-antimycotic) at 23°C as normal conditions. To induce the heat shock cells were incubated at 37°C for 1 h before further experiments.

Real-time quantitative PCR, RNA-seq, and data analysis

Cells were lysed on plates and total RNA was extracted. For RT-qPCR – the cDNA synthesis was performed. RNA extractions and cDNA synthesis from all samples were performed for three biological replicates.

RT-qPCR was performed using QuantStudio™ 5 thermocycler and data were

calculated by connected Applied Biosystems™ qPCR analysis module.

For RNA-seq – mRNA enrichment, library construction, and Illumina sequencing were performed (Novogene). Raw data were pre-processed, mapped, and analyzed using the DESeq2 analysis pipeline in RStudio.

Western blot/Immunofluorescence and analysis

Standard western blot/immunofluorescence procedure was performed and data were analyzed using Image Lab/ImageJ software.

Results

We have developed a protocol that allows us to study the stress response in cells.

We have found and functionally described large changes in global transcription in response to stress stimuli. Our data show that the level of some transcripts, widely considered as stable reference genes, alter after exposure to stress.

RNA-seq analysis allowed us to select the set of genes that remain stable in heat shock response and between different cell lines: Kc and S2.

We have shown that under stress the phosphorylation of HSF and lamin Dm occur. We also have shown one of a stress-dependent phosphorylation site in lamin Dm – Ser25.

Discussion and conclusions

Epigenetics is a field of the future. So far, many chromatin remodelers with histones and RNA polymerase II in front have been identified as those in which post-translational modifications either activate or lead to gene repression. Global transcription shut down is clearly visible in heat shock. In this study, we show that working on models such as stress response (and others causing global expression alterations), scientists should be very careful in choosing reference genes for normalization – even with genes widely considered as stable.

In our project, we show that during heat shock, specific phosphorylation of lamin occurs on Ser25, which results in a change in its solubility and potentially leads to stronger binding of chromatin in stress. These data may indicate that lamins play a key role in turning down gene transcription.

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Session 6, **O 7.**

Male infertility in context of oxidative stress: the analysis of Total Antioxidant Status and clusterin concentration in human seminal plasma – pilot study

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Background

Infertility becomes a significant problem around the world, especially in the developed countries. It is estimated that nearly 15% of couples of the reproductive age are trying to have a child without success. In Poland this problem affects about million and a half of couples, and the male factor alone contributes to about 50% of those cases [1, 2]. Despite the development of medical sciences, there is lack of early, sensitive male infertility biomarkers. Semen analysis, routinely performed during the male infertility diagnostics, may result in no pathological values. It concentrates mainly on the spermatozoa features. Ninety-eight percent of human semen constitutes seminal plasma which is a mixture of many proteins playing a relevant role in the proper function of the male reproductive system and fertilization process. Among them clusterin (CLU) is one of the important human seminal plasma glycoproteins, playing crucial role in the proper sperm cells maturation as well as in the maintenance of oxidative-antioxidative balance [3]. Among many reasons of male infertility, an oxidative stress is considered as one of the most important [4]. In this study, we decided to determine clusterin concentration and assess the Total Antioxidant Status (TAS), one of the oxidative-antioxidative balance parameters in seminal plasma of infertile men from astenozoospermic and teratozoospermic groups, in comparison to the results obtained for normozoospermic patients.

Material and Methods

Seminal plasma samples of 72 infertile male partners of the reproductive age were collected in the Laboratory of Infertility Diagnostics, Clinical Center of Gynecology, Obstetrics and Neonatology, Opole, Poland. The standard semen analysis was carried out according to WHO directives [2010] and based on its results, patients were classified as teratozoospermic (n=26), astenoteratozoospermic (n=19) and normozoospermic (n=27). The ejaculates were centrifuged (3500×g, 10 min, RT) to obtain seminal plasma. Seminal plasma clusterin concentration was determined using ELISA Kit (Human Clusterin, Bioassay Technology Laboratory). The Total Antioxidant Status was estimated in patients' seminal plasma using automatic method (Randox Laboratories Ltd.) in autoanalyser Konelab 20i®. Statistical analysis was performed using STATISTICA 13.3 PL (StatSoft Inc.) software (U Mann-Whitney test).

Results

The median value of clusterin concentration was visibly lower in the normozoospermic group in comparison to the teratozoospermic and astenoteratozoospermic patients. The reversed trend was observed for median of TAS concentration. However, no statistically significant differences in the values of determined by us parameters between analysed seminal plasma groups were observed.

Discussion and conclusions

Seminal clusterin concentration in men with decreased fertility/infertile seems to be an interesting factor when analysed in the context of oxidative-antioxidative balance. Further investigations, on larger number of patients, as well as the determinations of other oxidative stress parameters, are needed in this field.

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Session 6, **O 8.**

A different strategy to prevent protein adsorption

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Background

The nonspecific adsorption of proteins at various interfaces is well-known, undesirable phenomenon that could possibly lead to deterioration of the medical implants [1]. Given that nonspecific adsorption of proteins must be suppressed, different strategies for chemical modification of surface properties of the implants are constantly developed. These strategies can be divided into three main categories: covalent grafting of polymer brushes, surface initiated polymerization and chemisorption of thiol-terminated polymers. Despite the effort devoted to develop aforementioned strategies, their application is still limited mainly due to the need of harsh chemical usage. For this reason, in this work we present an alternative approach consisting of immobilization of poly(styrene/α-tert-butoxy-ω-vinylbenzylpolyglycidol) microparticles – P(S/PGly) in diffusion controlled, electrostatic-driven process.

Material and Methods

Polymer microparticles were synthesized in radical, surfactant-free emulsion polymerization in aqueous medium. After purification, the basic physicochemical properties of investigated microparticles such as hydrodynamic diameter and zeta potential were determined for broad range of pH using Malvern Zetasizer apparatus. Silicon plates modified by adsorption of poly(allylamine hydrochloride) were chosen as model surface utilized for streaming potential and adsorption kinetics studies. NT-MDT Solver Atomic Force Microscope (AFM) were used to determine both roughness of silicon plates and adsorption kinetics of microparticles. Antifouling properties of obtained surfaces were determined using streaming potential measurements. In this case, a monolayer of microparticles was obtained directly in the streaming potential cell and streaming current was determined. Afterward, the cell was flushed and filled with human serum albumin (HSA) solution. HSA was adsorbed under diffusion controlled conditions for certain amount of time. Afterward, the cell was flushed with NaCl solution and streaming potential was determined again.

Results

Firstly, the hydrodynamic diameter and zeta potential of microparticles were determined for pH range 4-10 and ionic strength equal to 10^{-2} M and 10^{-3} M. It was found that hydrodynamic diameter of microparticles is equal to 350 nm for both investigated ionic strengths. Moreover, it was found that hydrodynamic diameter does not depend on pH value. This is in agreement with diameters derived from SEM analysis. Performed zeta potential measurements proved that P(S/PGly) microparticles exhibit negative surface charge in whole investigated pH range. To be more precise, the zeta potential determined for ionic strength equal to 10^{-2} M diminished from -45 mV to -60 mV when pH changed from 4 to 10. This confirmed the possibility of P(S/PGly) microparticles immobilization on positively charged surface of PAH-modified silica. From adsorption kinetics experiments performed for different adsorption time (1-40 h) it was found that experimentally determined adsorption rate is twofold lower compared to the theoretical one. Moreover, it was

revealed that jamming coverage of investigated microparticles is significantly higher than maximum coverage predicted by RSA model for spherical, rigid spheres. Finally, HSA adsorption measurements revealed that compared to bare PAH/SiO_2 surface, the amount of adsorbed HSA is threefold smaller for PAH/SiO_2 surface modified by adsorption of P(S/PGly) microparticles.

Discussion and conclusions

Kinetic measurements performed for P(S/PGly) microparticles immobilization at PAH/SiO_2 surface enabled to determine the structure of investigated microparticles. It was found that effective density of such particles was equal to 0.6 g ml^{-1} which is considerably lower that density of typical polystyrene microparticle (1.05 g ml^{-1}). This results from the presence of polyglicidol-rich, fuzzy shell layer, which thickness was estimated to be equal to 25 nm. The structure of investigated particles was confirmed both by streaming potential and AFM measurements. Streaming potential method enabled also a determination of anti-fouling properties of particle monolayers. It was confirmed that for modified surfaces, the adsorption of HSA was noticeably lower (0.4 mg m^{-2}) than in the case of bare one (1.3 mg m^{-2}) [2]. This effect can be attributed to the presence of fuzzy, shell layer with properties similar to polymer brush. In such case, the protein/surface interaction energy is significantly lower due to prevention of HSA molecules from approaching the surface and thus forming a strong, electrostatic-driven interaction. One can therefore expect that immobilization of P(S/PGly) microparticles can be efficient strategy for creating controlled, protein-repelling surfaces.

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Session 6, **O 9.**

Volatile compounds as a means of protecting bacterial contamination of cosmetics

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Background

Microorganisms are omnipresent, also occupying our skin, because of that cosmetics are often contaminated. Preservation of everyday use products is important field of cosmetic industry. Moreover in everyday use cosmetics are contaminated by incorrect application. All cosmetic products should be tested on presence of pathogenic and spoiling microorganisms. Due to recent trend in minimalizing chemical substances in cosmetic formulation, new antimicrobial substances of natural source are sought. Furthermore possibility of combining fragrance properties with antimicrobial activity seams promising.

Material and Methods

Fifty-three volatile carbonyl compounds and its oximes were tested. Carbonyl compounds were obtained from Merck Poland except *trans*-cinnamaldehyde, α-hexylcinnamaldehyde, *p*-tolualdehyde, piperitone

that were obtained from Tokyo Chemical Industry Co. LTD, piperonal that was obtained from LOBA Chemie Austria and vanillin that was obtained from Avantor Performance Materials Poland S.A. All corresponding oximes were synthetized in our laboratory. The correctness of oxime structure was confirmed by GC-MS and NMR and will be presented in further studies. All over hundred substances were diluted in DMSO to the final concentration of 30 mg/mL. Microorganisms used in this study represent product spoiling species commonly associated with beauty care products. Tested microorganisms were obtained from Mecconti S.A.R.L. Sp. z o.o and consisted: Gram negative: *Enterobacter gergoviae, Klebsiella aerogenes, Burkholdria cepacia* Gram positive: *Kocuria rhizophila, Staphylococcus epidermis.*

Results

The best results was obtained by *trans*-cinnamaldehyde, citral, *trans*-cinnamal aldehyde oxime and saphranal oxime against *K. aerogenes*, the MIC value was 300 μg/mL. Inhibitory activity of other test agents was not satisfactory.

Discussion and conclusions

Among all tested carbonyl compounds only one – pseudoionone is excluded from use in cosmetics. Citral, hexyl cinnamaldehyde and α-isomethylionone are on list of subject to the restrictions according to Regulation (EC) No 1223/2009 of the European Parliament and of the Council of 30 November 2009 on cosmetic products. Presented results shows that none of tested substances are appropriate to be used as preservatives in cosmetic formulation exposed to tested microorganisms. Although those aroma compounds might enhance activity of more familiar preservatives. Further test on other spoiling microorganisms are necessary.

Session 6**, O 10.**

Studies on the reactivity of human serum IgG and IgA antibodies with the bacterial OmpC protein as a potential diagnostic marker of humoral immunodeficiency in children

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Background

OMPs (outer membrane proteins) are immunologically important bacterial components because they are mainly exposed on the cell surface and may affect the physiological functions of host tissue, contributing markedly to the mechanisms of pathogenicity, progression of infection and the development of inflammatory response. The OmpC is a major protein in enterobacterial outer membrane recognized by human immune system. Accessibility of OmpC to host defense mechanisms make them attractive as immunodiagnostic markers, potential immunoprophylactics and convenient carriers for carbohydrate antigens. Preliminary research performed on mice

have showed that OmpC protein from *Shigella flexneri* 3a plays a protective role against enterobacterial infections [2]. The reactivity of OmpC with human sera revealed low total levels of IgG and IgA in immunodeficiency in children [1]. The peptide (RYDERY sequence) was identified as an epitope recognized by antibodies, indicating the OmpC may serve as an immunodiagnostic marker [2]. Moreover, the peptide with OmpC epitope sequence can be used for binding to carrier proteins for better availability antigen for induction of specific antibodies.

Material and Methods

Sera of patients have been obtained from Medical University of Wrocław. The OmpC was isolated from *Shigella flexneri* 3a (PCM 1793) by valeric acid method and purification with gel filtration and ion exchange chromatography. BSA (bovine serum albumin) conjugates with linear/cyclic peptide of epitope sequence from OmpC protein was prepared as described [2]. IgG and IgA levels in sera of patients were measured by ELISA assay [1-3].

Results

Degree of BSA substitution with peptides was estimated by MALDI-TOF-MS. The OmpC protein and BSA-peptide conjugates were analysed with SDS-PAGE/immuno blotting assay to check its purity and immunoreactivity with human serum samples. The OmpC and peptide conjugates were used in ELISA. The IgG and IgA antibody levels were determined in sera of immunodeficiency patients and in children with recurrent respiratory tract inflammation, of which the level was compared to the healthy controls.

Discussion and conclusions

Results of ELISA assay show that the reactivity of both IgA and IgG antibodies with enterobacterial OmpC protein was significantly lower in immunodeficiency children than in healthy children and adult blood donors and increased gradually with age, although values for IgA were more distinct than those of IgG. The heterogeneous correlations of specific total IgA and IgG antibodies may come from the various ages of child patients and different deficits of specific antibodies depending on the status of immunoglobulinopathy. However, the results of this study are promising and suggest that the OmpC from *Shigella flexneri* 3a might serve as a specific humoral immunodeficiency marker, especially in IgA deficiency and recurrent respiratory tract infections.

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POSTER PRESENTATIONS

The molecular study of anticancer activity of novel, synthetic derivatives of naringenin

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Background

Flavonoids belong to a large group of natural compounds found in many plants, where they play an important role in cell protecttion, fruit coloring, photosensitization, and plant growth regulation. Numerous investigations have confirmed their variety of biological activities such as anticancer, antiinflammatory, antioxidant, etc [1,2]. Naringenin (4', 5, 7-trihydroxyflavone) is an active form of naringin, a substance commonly found in citrus fruits. The biological activity, easy accessibility and low cost of the extraction make naringenin an attractive candidate for anticancer therapy.

We have previously shown that novel, synthetic O-alkyl derivates of naringenin and their oximes can act as effective antitumor agents [3]. The aim of this study is to perform the molecular analysis of their anticancer activity.

Material and Methods

The cytotoxic effects of examined compounds on various cancer cell lines were measured using sulforhodamine B assay. Phosphatidylserine exposure and membrane integrity were investigated using RealTime-Glow™ Annexin V Apoptosis and Necrosis Assay (Promega). The activity of Caspase-3 and -7 was analyzed by Caspase-Glo3/7 Assay (Promega). The detection of procaspases and its active form was performed using western blot method. Nuclear DNA was extracted from cells and analyzed by electrophoresis to detect DNA fragmentation. Staurosporine was used as a reference agent for the induction of apoptosis.

Results

The results indicate that different type of cancer cells are similarly sensitive to all investigated compounds except for naringenin and staurosporine.

The apoptosis/necrosis assay shows that cytotoxic effect of alkyl derivatives of naringenin is caused by the necrotic pathway. The substitution of the oxime group to these compounds induces phosphatidylserine exposure and cellular membrane disintergration. The results confirm that oxime 7-O-decylnaringenin activates apoptosis in HT29 cells, although time between the induction of phosphatidylserine exposure and the loss of membrane integrity was over 4 times shorter than observed for staurosporine. In contrast, oxime 7,4'-di-O- butylnaringenin simultaneously induces signals from both annexin V and DNA dye. This suggests that the activated process differs from apoptosis.

On the other hand, following 7,4'di-O-butylnaringenin exposure, we observed an activation of the caspase pathway which was manifested by the increased reactivity of cleaved forms of caspase-3 and -7.

Interestingly, the increases of caspase -3 and -7 activities are detected 4 hours after treatment of the cells. A similar effect is observed for oxime 7-O-decylnaringenin, but the time of caspase activation is longer than 12 hours. The analysis of caspase 3 and-7 supports hypothesis that mechanism

of action of naringenin and its alkyl derivatives differs from apoptosis.

One of the last step of cell apoptosis is DNA fragmentation. To verify the hypothesis that oxime derivatives can induce the apoptosis process, we performed an examination of genomic DNA, extracted from HT29 cells after 48-hours of treatment with studied compounds. The results prove that only staurosporine can activate DNA degradation in the cells.

Discussion and conclusions

Our data show that despite the activation of effector caspases 3 and 7, the mechanism of action of oxime 7,4'-di-O-butylnaringenin and oxime 7-O-decylnaringenin differs from typical apoptosis. Further investigation should be carried out in order to clarify whether we have observed a new type of cancer cell death or overlapping effects of apoptosis and necrosis.

This project was financed by the National Science Centre of Poland, Grant No. 2016/21/B/NZ9/01904.

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Changes in myosin light chains expression in the mechanism of adaptation to oxidative stress

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Background

Myosin consists of two heavy chains (MHC-α and MHC-β) and two pairs of light chains: essential (ALC1 – atrial and VLC1 – ventricular) and regulatory (RLC). Oxidative stress associated with heart diseases leads to disruption of the balance between synthesis and degradation of contractile proteins – it has been shown to induce post-translational modification of myosin light chains, making them more susceptible to degradation by metalloproteinase 2 (MMP-2). This results in the degradation of VLC1 in heart ventricles and the increased expression of ALC1 instead. The aim of the study was to investigate changes in the expression of myosin light chains in rat cardiac myocytes under the influence of increased oxidative stress. This study may be an introduction to further research of cardiac function and actin-myosin interaction, as a result of the replacement of VLC1 for ALC1 during ischemia/reperfusion (I/R) injury.

Material and Methods

Wistar rats hearts were perfused using the Langendorff method. The first group – I/R – underwent oxygen stabilization (25 min), global ischemia (22 min) and oxygen reperfusion (20 min). Aerobic control was only introduced to aerobic conditions for 77 minutes. Next atria were cut off from the hearts and ventricles were taken for analyses. In 47th minute of the experiment coronary effluents were collected from the buffer flowing through the heart. Lactate dehydrogenase (LDH) activity was measured in the coronary effluents to examine cardio-

myocyte damage after the ischemia. RQ-PCR was performed for ALC1 gene. Quantitative analysis of ALC1, VLC1 and MMP-2 proteins was performed using ELISA tests. MMP-2 activity in heart homogenates was assessed by gelatin zymography.

Results

LDH activity was significantly increased in I/R group in comparison to Aero group (p=0.01). Expression level of ALC1 gene was significantly higher in I/R group in relation to Aero group (p=0.004). ALC1 protein content in hearts homogenates was also significantly increased in I/R group in comparison to Aero group (p=0.03). VLC1 content in coronary effluents was substantially increased in I/R group in comparison to Aero group (p=0.02), which confirmed that VLC1 was released into extracellular space. MMP-2 concentration in heart homogenates was increased in I/R group in comparison to Aero group (p=0.03). The activity of pro-MMP-2 and active-MMP-2 forms was also increased in I/R group in comparison to Aero group (p=0.04, p=0.03, respectively). Accordingly, the total-MMP-2 activity was higher in I/R group in comparison to Aero control (p=0.03).

Discussion and conclusions

In conclusion, above data showed that ischemia and reperfusion induced changes in VLC1/ALC1 already at the level of gene expression. There are many studies fascinating in VLC1/ALC1 replacement but the researchers consider different heart diseases, for example progressive heart failure, ventricular aneurysmectomy, familial hypertrophic cardiomyopathy [1, 2]. In general they confirmed the existence of VLC1/ALC1 replacement mechanism to improve heart function [3, 4]. This preliminary study provides the basis for further studies on cardiac function changes, actin – myosin interactions and an influence of MMP-2 on contractile proteins.

This work was supported by the National Science Centre [grant no. UMO-2016/23/B/NZ3/03151].

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The unique content of oat oil – the perspectives of exploitation

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Background

In recent years, there has been an increase in consumer awareness of the benefits resulting from consuming oat-based products [1]. The main advantage of oat products is their good perception among consumers which increase the willingness to buy. Oats contain many antioxidant compounds (polyphenols, avenantramids, tocochromanol, phytic acid, MUFA and PUFA, including α-linolenic acid, melatonin, inositol phosphates, phyto-

sterols) and other biomolecules of well recognized health impact (β-glucans) [2-4]. Compared to other major cereals like barley, wheat and rice, oats are still underestimated crop [5]. Research continuously proves that oats are very interesting plant, which composition offers a variety of substances that may in the future be used in the food, pharmaceutical, cosmetic and chemical industry [6-8]. Still unexploited oat oil contains a large amount of polar lipids compared to other oilseeds [9]. In the future, polar lipid fractions may be widely used in the stabilization of emulsions used in the food industry e.g. for the production of chocolate, lubrication and baking pastes [10-11].

Discussion and conclusions

The oil contained in oats has not been used much so far, but the development of new methods and techniques of lipid fractionation allows to use its potential in many industries. Polar oat oil lipids such as glycolipids and phospholipids contain hydrophilic and lipophilic groups in their structure.

A change in the balance between these two groups enables modification of emulsifying properties of these lipids and affects the stability of emulsions [12].

The most numerous phospholipids in oat oil are phosphatidylcholine (PC) and phosphatidyl ethanolamine (PE) [13], digalactosyldiacylglycerol (DGDG) is the glycolipid present in the highest amount [14]. In current dietary trends, the composition of food products becomes a key factor for the consumer. Such approach forces industry into, for instance, choosing the appropriate emulsifier which then affects stability and then the product quality [15]. The need for food diversification on the market is forcing the search for a new solutions for food preservation, which also starts the demand for emulsifiers with new properties. The answer could be the new emulsifiers from polar lipids extracted from underestimated raw materials like oats. Due to the characteristics of the process of obtaining oat oil and low allergenicity of oat in total, no allergic risk is foreseen as is the case of emulsifier of soy or chicken eggs origin [16].

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Oat oil samples from polar and non-polar extraction characterisation with near (NIR) and medium infrared (MIR) spectroscopy

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Background

The oil contained in oats has not been used so far; however, the development of new methods and techniques of lipid fractionation creates new possibilities and expands the use of particular oat oil components in the food industry [1], cosmetic [2] and pharmaceutical industry [3]. The research proves that oats are an interesting plant, containing in its rich composition a variety of substances that may be used in the food industry in the future, e.g. in creating new solutions for exclusion diets or functional food products [4]. The development of new methods and techniques of lipid fractionation allowed to separate such components from oat oil, which have huge application potential and have not been used before [5].

Material and Methods

The oat oil was obtained by Soxhlet continuous extraction. The extraction was proceeded with two different solvents – ethanol (99.8%) and petroleum ether (40/60) and extraction time was 8h. The obtained oil samples were tested in near (NIR) and medium infrared (MIR). The oil spectra were measured on the Nicolet 6700 FT-IR spectrophotometer using the high-performance diamond SMART iTX ATR accessory, and the NIR measurement accessory.

Results

Two different solvents used during extraction provided two distinct oil samples. Ethanol extracted oil sample was orangeish while petroleum ether extraction resulted in yellow-olive colour of the oat sample. The next easily observed difference was noted in the consistency of obtained oil samples. Polar extraction sample (ethanol) was notably thicker, and maintained in room temperature started to solidify while rising the temperature caused liquefaction of sample.

Non-polar extraction with petroleum ether provided liquid sample which only started to solidify when stored in 4 [°C]. Different aroma was noted for oat oil samples being pleasant "bread-like" for polar extraction sample and oat aroma mixed with petroleum scent for non-polar sample.

The spectra obtained with different solvents shows specific differences, and especially at 3500 cm^{-1} the sample extracted with polar solvent can be easily distinguished.

Discussion and conclusions

The differences observed in the samples of oat oil obtained are due to the difference in polarity of the solvents used for extraction and the associated different extraction capacity. The petroleum ether, which is non-polar in nature, extracts mainly non-polar lipids and the colouring matters responsible for the greenish colour of the sample. Polar ethanol isolated the polar fraction of lipids, i.e. phospholipids and glycolipids, which caused the sample to thicken at room temperature, as opposed to the sample obtained by extraction with petroleum ether.

Extraction with Soxhlet's apparatus using two different solvents allowed to isolate different components from oats depending on the molecular structure affecting their polarity and properties.

The Near Infrared (NIR) and Medium Infrared (MIR) spectroscopy method allows for a quick qualitative evaluation of oat oil.

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Myelin as an example of lyotropic liquid crystal

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Background

Lyotropic liquid crystals can be observed in complex biological systems, for instance in cell membrane and myelin sheath. Both consist of components with unique ability to create bilayer structures – lipids. They are amphiphilic molecules which tend to self-organize into lamellar structure in aqueous conditions. Myelin, in contrast to most biological membranes, exhibits high ratio of lipids to proteins. This structure is a target of various autoimmune diseases, therefore it is crucial to be able to analyse and visualise fine changes of its arrangement [1]. Moreover, there are several possibilities to obtain artificial myelin, which can be use as model of myelin sheath [2]. Herein, we present two formation methods of myelin figures.

Material and Methods

In this study, we used commercially available 1,2-dilauroyl-sn-glycero-3-phosphocholine (DLPC). First, to prepare myelin figures, a dry droplet of phospholipids was hydrated. Thus, growth of myelin tubes could spontaneously occur on the edge of lipid plaque in high magnification. The elongated structures were observed under the polarized light microscope. Additionally, we performed an experiment with a slowly evaporating droplet. Myelin growth was observed at the contact line between the droplet and a barrier [3].

Results

Hydration of dry lipid plaque caused growth of myelin figures. As shown in Fig. 1, they are formed from the edge of the dry droplet. This material can be observed under crossed polarizes, which means that the tubes of lipid bilayers exhibit *birefringence*. Moreover, research which were performed with a retardation plate showed that myelin tubes are three dimensional structures. In contrast to results obtained by the first experiment, the second experiment on the system with slow water evaporation, gave smaller diameter of myelin figures and was more sensitive to temperature changes.

Discussion and conclusions

Two different ways to obtain myelin tubes are shown. Each experiment allows us to obtain different types of elongated myelin structures. Presumably, quality and parameters of prepared material depend on

a factor which causes growth of tubes. Furthermore, artificial myelin could potentially be used as a model in biomedical applications to mimic *in vivo* behaviour of myelin sheath.

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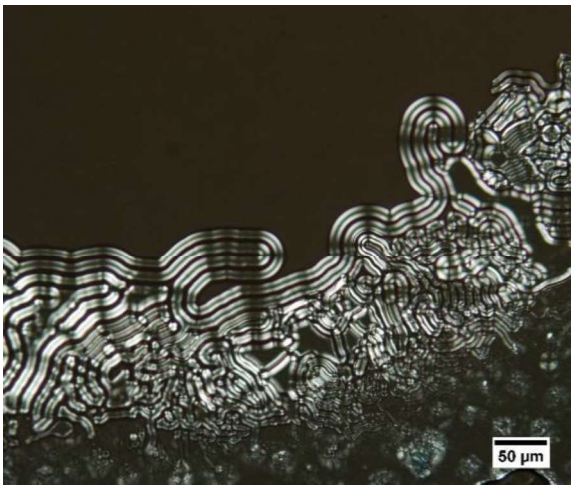

Fig.1. Myelin figure under the polarized light microscope. Scale bar 50nm

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Preliminary view on *Danio rerio* and *Homo sapiens* otolin-1

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Background
Mineralization of variable tissues in invertebrates and vertebrates is a strictly regulated process which involves action of many distinctive intra- and extracellular proteins [1]. These proteins control the deposition of inorganic material [2]. Biomineral matrix proteins are a wide variety group in which intrinsically disordered proteins as well as proteins structurally defined can be distinguished [1]. Here we present preliminary comparison of molecular properties between recombinant *Homo sapiens* and *Danio rerio* collagen-like otolin-1 [3].

Material and Methods
Recombinant protein expression and purification Danio rerio (dOtolin1) and *Homo sapiens* (hOtolin1) otolin-1 was expressed in Arctic Express *Escherichia coli* in pQE80L plasmid for 24 h in 16°C. Protein purification included immobilized metal affinity chromatography (IMAC) and size exclusion chromatography (SEC).

Circular dichroism spectroscopy

Far UV CD spectra was recorded with Jasco J-815 spectropolarimeter at 20°C between 260 and 200 nm with scanning speed of 20 nm/min every 0.5 nm with 3 accumulations.

Nano-Differential Scanning Fluorimetry

Thermal shift assay was performed in presence of variable calcium ions concentrations with Prometheus device (NanoTemper). The range of temperatures was between 20°C and 110°C with the linear increase of 5°C/minute.

Limited proteolysis

To analyse the susceptibility of specific proteolysis of dOtolin1 and hOtolin1 the time-limited proteolysis of both proteins was performed in presence of V8 protease at 23°C in time periods between 10 and 210 minutes.

Results

Recombinant protein expression and purification

The proposed way of recombinant dOtolin1 and hOtolin1 resulted in yields of 1 mg and 5 mg of protein per 1L of bacterial culture respectively.

Circular dichroism spectroscopy

The estimation of dOtolin1 and hOtolin1 secondary structure content by CDPro shows differences in helical content between them (in case of dOtolin1 10% higher than in case of hOtolin1).

Nano-Differential Scanning Fluorimetry

The presence of calcium ions increases the thermal stability of both proteins. hOtolin1 is more sensitive on calcium ions concentration. The first denaturation midpoint is increased from 41°C (10mM EDTA) to 68°C in presence of 0.1mM Ca^{2+}. In case of dOtolin1 transition temperature is affected by 1mM Ca^{2+} concentration and is equal to 86.7°C.

Limited proteolysis

The outcome of the assay shows the difference of the susceptibility of proteolytic digestion with V8 protease of dOtolin1 and hOtolin1. dOtolin1 is not affected by the incubation with V8 protease for 3.5 hours. By contrast, hOtolin1 first degradation products occur in 45 minutes after the start of incubation.

Discussion and conclusions

Danio rerio otolin-1 and *Homo sapiens* otolin-1 are originated from two distantly related species, yet they fulfil homologous functions providing the scaffold of fish otolith and human otoconia. The preliminary analysis of dOtolin1 and hOtolin1 shows differences in their molecular properties. The amount of helical content between short collagen-like dOtolin1 and hOtolin1 differs. This difference can be an explanation of the resistance of proteolytic lysis with V8 protease, where dOtolin1 is less affected by the action of the protease. Additionally, the thermal stability of hOtolin1 is stabilized by lower concentrations of calcium ions in comparison to dOtolin1. In future, the analysis of the influence of post-translational modifications on the properties of otolin-1 is planned.

Acknowledgments:

This work was supported by the National Science Center (Poland) [UMO-2015/19/B/ST10/02148] and in a part by statutory activity subsidy from the Polish Ministry of Science and High Education for the Faculty of Chemistry of Wroclaw University of Science and Technology

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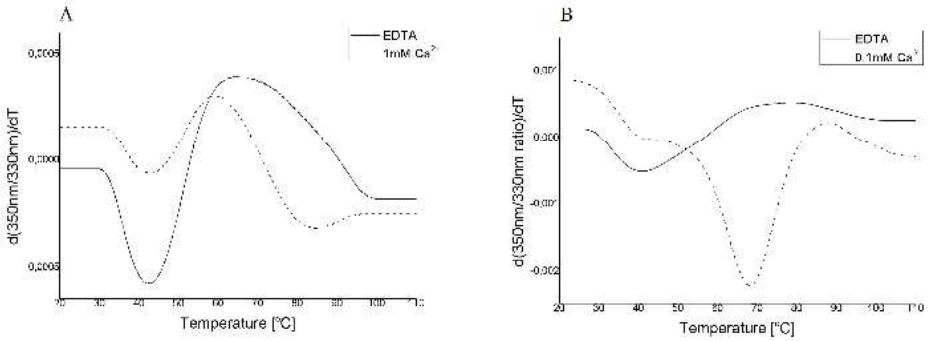

Fig. 1. The denaturation midpoint of dOtolin1 (A) and hOtolin1 (B) at a given EDTA and Ca^{2+} concentration presented as the first derivative of the ratio between intensity of fluorescence at 350 nm and 330 nm in respect to temperature

Mitochondrial proteome changes by doxycycline may protect organ graft against perfusion injury

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Background

End stage renal disease is the final phase of chronic kidney disease. It is well known that hemo- or peritoneal dialysis is life-saving in patients who progress to chronic renal failure [1]. In North America, approximately 75% of all solid organ transplants performed are kidney transplants [2]. The transplantation organ from one person to another is necessarily accompanied by injury occurred during either warm or cold ischemia.

The preservation of kidneys for transplantation relies mainly on hypothermia to decrease cellular metabolism and conserve stores of adenosine triphosphate. Because metabolism is ongoing, albeit at a slower rate, the duration of cold ischemia should be minimized as much as possible [3]. Even with machine cold perfusion, significant preservation injury nonetheless occurs, and likely contributes to delayed graft function and acute tubular necrosis in the transplanted kidney [4]. Injury of any cause is suspected to lead to a decrease in kidney function, shortened graft survival, and an increase in rejection due to increased activation of the immune system [5].

It has been previously shown that doxycycline (Doxy) protects the kidney from preservation injury by inhibition of matrix metalloproteinase. However, the precise molecular mechanism involved in this protection from injury is not known. For this reason the aim of the current study was to

assess the potential mitochondrial target for doxycycline nephroprotection.

Material and Methods

Male Sprague-Dawley rats were used as a surrogate model of *ex vivo* kidney perfusion. The left renal artery was ligated in situ for 10 minutes of warm ischemia, then cannulated and the kidney was removed and rapidly cooled to 4°C. The kidney was perfused with a standard perfusion buffer with the addition or without doxycycline (100 μM) for 22 h. Then, tissue, protein extract from kidney and perfusates were analysed by EM, 2DE, MS and biochemical tests. Graphpad Prism v. 6.0 was used for statistical analysis.

Results

LDH, NGAL and total protein levels were measured in perfusates as the markers of injury. A significant increases in LDH activity and NGAL levels were observed in perfusates from ischemic kidneys compared to the controls. 100 μM Doxy decreased cells injury during cold perfusion ($p<0.05$). Electron microscopy confirmed, that doxycycline protected the kidney from the separation of cells and enlarging of the extracellular space, as well as from the formation of dense bodies and mitochondria damage (fig. 1) Analysis of kidney homogenates by 2DE and identification by mass spectrometry revealed proteins such as N(G),N(G)-dimethylarginine dimethylaminohydrolase, and phosphoglycerate kinase 1 were increased by Doxy in comparison to the controls.

Discussion and conclusions

This study allowed to get the knowledge about the specific mechanism by which inhibition of MMPs protects kidneys from cold preservation injury. Data showed that the maintenance of mitochondrial metabolism and mitochondrial structure was the main target of doxycycline nephroprotection.

Study was supported by the National Science Centre grant no. UMO-2017/27/B/NZ4/00601

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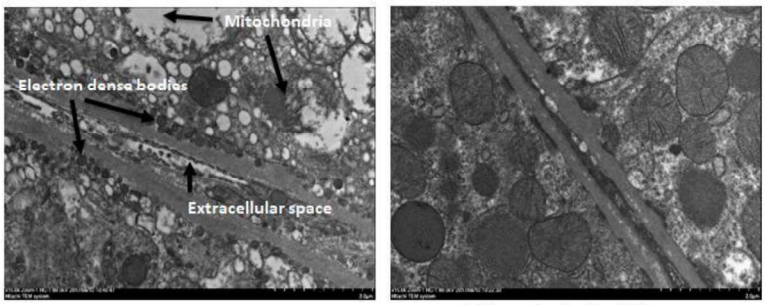

Fig.1. Representative electron micrographs of control rat kidneys (left micrographs) and perfused with Doxy (right micrographs). 1500x magnification, Doxy – doxycycline

In silico of study of imidazole based compounds as potent inhibitors of p53-MDM2 interaction

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Background

It is widely known that p53 protein influence many cell processes, including activation of DNA repair or induction of apoptosis [1]. The interaction of MDM2 with p53 is the most common reason of its inhibition what disrupt many signaling pathways. The detailed mechanism of this phenomenon is not fully understood. Structural findings proved that Leu26, Trp23 and Phe19 sequence in tumor suppressor protein is the key factor. Last studies provide also suggestions that inhibition of p53-MDM2 interaction leads to activation of p53's tumor suppressing function [2]. Therefore, the p53-MDM2 interactions are extensively studied in terms of anticancer agents design [1].

Material and Methods

Computer-aided drug design become an essential tool to discovery and analyze compounds of the potential therapeutic applications. In the present project we used computational approaches in order to design of p53-MDM2 interaction inhibitors. The structures of proposed ligands were optimized at the B3LYP/6-31G** level of theory. The molecular docking was performed for compounds based on imidazole scaffold and the human MDM2 protein originated from the Protein Data Bank (PDB ID: 3LBK) [3].

Results

Table *The values of free energy of binding and inhibition constant for most potent compounds.*

Compound	Free energy of binding [kJ/mol]	Inhibition constant
A	- 36	765 nM
B	- 35	821 nM
C	- 31	4 μM
D	- 31	4 μM
E	- 31	3 μM
F	- 35	843 nM

The most potent compound can bind to the hydrophobic cavity of MDM2 and interact similar to p53 (binding energy -36 kJ/mol). Phe55, Leu57, Ile61, Tyr67, Phe91, and Ile99 are the main amino acid residues involved in hydrophobic interactions. Additionally, the best inhibitor form hydrogen bond with Leu54 (See Figure and Table).

Discussion and conclusions

In the present study we predicted the nature and strength of binding of imidazole derivatives to MDM2 protein as a target. In accordance with the previous study the proposed imidazole derivatives are able to bind to the same binding cavity of protein but shows better inhibition constants than tested before[1].

Acknowledgments

Work was partially supported from SUB.0080.19029

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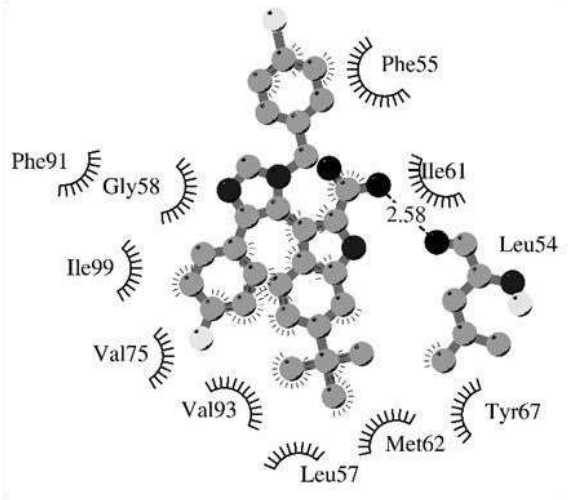

Figure Intermolecular interactions between compound A and human MDM2 protein. The hydrophobic forces are shown as arches and the hydrogen bonds as a line

The impact of xenoestrogens on the effectiveness of treatment of hormone-dependent breast cancer

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Breast cancer is the most common cancer among women and also causes the highest number of cancer-related deaths in women [1]. In the treatment of hormone-dependent breast cancers, the most important is hormone therapy, including tamoxifen, aromatase inhibitors and their sequence [2]. In the pathogenesis of breast cancer xenoestrogens, as an exogenous substances which can interfere with the functioning of the endocrine system, is the subject of many studies. Numerous publications confirm that many compounds commonly found in the environment can act as modulators of estrogen receptors and thus compete or mimic the action of endogenous estrogens (e.g. stimulate the proliferation of cancer cells). This was confirmed in *in vitro* and *in vivo* tests for i.a. bisphenol A [3] and metalloestrogens [4]. Data on phytoestrogens are still unclear [5]. Definitely less is known about the impact of xenoestrogens on the effectiveness of hormone therapy used to treat breast cancer, and possible drug-xenoestrogen interactions.

A systematic review of the literature derived from PubMed, Embase, and Scopus, relating to xenoestrogens in the context of interactions with drugs used in breast cancer hormone therapy was performed.

Phytoestrogens, in particular genistein, are the best studied xenoestrogen group. This is because phytoestrogens are often used to control menopausal symptoms that occur in patients who are receiving hormone therapy. Interaction between tamoxifen and genistein in a postmenopausal breast cancer model was demonstrated. Adding low concentrations of genistein to tamoxifen causes a reversal of its therapeutic effect (inhibition of cell proliferation and arrest of the cell cycle in the G1 phase) [6]. Similar conclusions can be drawn from other studies, which showed that genistein at low doses suppresses the therapeutic effect of tamoxifen. Importantly, high doses of genistein do not suppress drug efficacy. The majority of supplements used to relieve menopausal symptoms are rather multi-component preparations containing several phytoestrogens whose effect may accumulate. The effects of both genistein and 8-prenylnarygenin alone, as well as four market-based, multi-component dietary supplements, on the effectiveness of 4-hydroxytamoxifen and letrozole were studied. Both genistein, 8-prenylnarygenin and all tested supplements have been shown to activate an estrogen receptor-dependent increase in MCF-7 cell proliferation that has not been inhibited by either 4-hydroxytamoxifen and letrozole [7]. Other studies, revealed that letrozole was shown to be effective in inhibiting tumor growth in mice, however, this effect was inhibited by the presence of genistein [8]. Examination of the effect of xenoestrogens present in the diet (genistein, zearalenone) on the effectiveness of letrozole and palbociclib treatment using the MCF-7 and T47D breast cancer cell lines showed that the combination of letrozole and palbociclib effectively inhibited the proliferation of cancer cells, while the addition of both genistein and zearalenone counteracted this effect [9]. Bisphenol A (BPA) is one of the best-tested for interactions with drugs used to treat breast cancer. It has been shown that with the simultaneous use of 4-hydroxytamoxifen and bisphenol A, the therapeutic effect of 4-hydroxytamoxifen decreases. This effect was greater the higher the BPA concentration [10]. Another xenoestrogen – methylparaben, also contributes to the occurrence of chemoresistance to drugs used in the treatment of breast cancer (tamoxifen, fulvestrant) [11].

Due to widespread exposure to xenoestrogens, as well as a steady increase in incidence of breast cancer, examining the impact of endocrine active compounds on the effectiveness of therapies used in the treatment of hormone-dependent breast cancer, is becoming a clinically important issue. As shown in this literature review, the majority of research focused on phytoestrogens. When analyzing the current state of knowledge, it seems that their intake should be avoided during ongoing cancer treatment. An area requiring further research is the analysis of the impact of xenoestrogens other than phytoestrogens, e.g. metalloestrogens, on the effectiveness of drugs used in the treatment of breast cancer.

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Antimicrobial activity of thyme, tea tree and eucalyptus essential oils against *Staphylococcus aureus* biofilm

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Background
Microbial biofilm is responsible for plethora of nosocomial infections [1]. Moreover, increasing resistance of microorganisms to antibiotics, forces search for alternative methods of infection treatment. Essential oils (EOs), which are compounds of high antimicrobial potential, are proposed as one of the possible solution of aforementioned issue [2, 3].

The aim of the study was to determine the activity of volatile and liquid phases of selected essential oils against S*taphylococcus aureus* bacterium.

Material and Methods
Three commercially-available essential EOs: eucalyptus (*Eucalyptus globulus),* thyme (*Thymus vulgaris*) and tea tree (*Melaleuca alternifolia*) were scrutinized with regard to their activity against *Staphylococcus aureus* ATCC 6538. To determine Minimal Inhibitory Concentration (MIC) and Minimal Biofilm Eradication Concentrations (MBEC) of aforementioned oils, serial dilution method was performed. In turn, volatile phase's activity against staphylococcal biofilm was assessed using a self-developed test method.

Results
Results revealed that MIC for eucalyptus, thyme and tea tree oil were 12,5%; 0,02% and 6,25% respectively, while MBEC values for these oils were >50%; 0,18% and 25% respectively. In case of analysis of volatile fractions, eucalyptus, thyme, and tea tree oil reduced 60%; 80% and 70% of staphylococcal biofilm, respectively.

Discussion and conclusions
EOs applied in this research display low toxicity, broad spectrum of effectiveness, biodegradability, immune-stimulating and anti-inflammatory properties [4,5].

Moreover, the results obtained suggest that application of essential oils against staphylococcal biofilm may be considered effective approach. Not only the liquid but also the volatile phases of all EOs have shown high efficacy against staphylococcal biofilm. The results concerning the activity of EOs liquid phase are confirmed by numerous

scientific reports [6, 7], while data on activity of Eos volatile phase is still scanty [8-10]. Therefore this study presents another step towards search of new treatment options directed against staphylococcal biofilms.

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What is hidden in hop cones?

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Background

Xanthohumol (XN), a prenylated chalconoid, is a natural product found in the female inflorescences of Humulus lupulus, also known as hops. Besides XN, hops inflorescences also contain flavanones like isoxanthohumol (IX) and 8-prenylnaringenin (8-PN), but at 10- to 100-fold lower concentrations than XN, respectively. All three substances are phytoestrogens: they naturally occur in plants and exert, directly or through metabolic changes, estrogenic effects because of structural similarity to 17β-estradiol [1,2]. Aim of the paper was to analyze the latest research applying 8PN as a phytoestrogen.

Material and Methods

Data was collected by anlyzing available articles which present results of studies related to 8-PN, XN and its association with phytoestrogen. Data were sought by computer-based searches from databases including PubMed, Google Scholar. Chosen literature represent researches conducted between 1999 and 2020.

Results

Around 35-40 years of age, women notice a physiological decrease in the production of their own female sex hormones. Their level, which decreases with age, initially results in insignificant, and then increasing, so-called traumatic symptoms of menopause such as fatigue, irritability, problems with concentration and memory, sleep quality deteriorates, hot flashes, dizziness and headache, trembling hands and occurring palpitations. Ailments can become a cause of professional absence, a decrease in productivity and quality of work, as well as a deterioration in the quality of life for women and their family members.

The average diet of European women contains too little phytoestrogens to show their beneficial therapeutic effect. Usually, daily intake is 1-3 mg of phytoestrogens, while women living on the Asian continent consume, on average, ten times more. The high content of phytoestrogens in the diet of Asian women results in a decrease in their incidence in the perimenopausal period and the lack of osteoporosis after the menopause.

Conclusions

Supplementation of phytoestrogens in the perimenopausal and postmenopausal period seems to be the right choice due to health and socioeconomic benefits. Hence the therapeutic treatment of 8-PN seems to be very promising because it has been described as the most potent phytoestrogen found in nature [3].

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Single treatment with Decitabine results in delayed morphological changes

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Background

Decitabine (5-aza-2'-deoxycytydine) is a hypomethylating agent approved for many haematologic malignancies treatment [1]. It is also established that it can successfully sensitize colorectal cancer (CRC) cells for topoizomerase inhibitors in combination therapy [2]. Even though the drug is in use for more than 40 years, its mode of action is not fully examined yet. Previous study showed that despite no direct cytotoxicity in colorectal cancer cells decitabine has more significant impact in prolonged culture. During 13/20 days of culture among observed changes were: induction of many morphological abnormalities, increase of p21 expression and reduction of cells clonogenity. All of those may inform about cell cycle inhibition connected to epithelial-mesenchymal transition or senescence.

The aim of this study was quantitative measurement of morphological changes of CRC cells long-term culture after one-time decitabine treatment and study the molecular events underlying the change.

Material and Methods

HCT116 cells were incubated with 0,25μM decitabine for 5, 7 and 14 days in standard culturing conditions. Afterwards cells were fixed and stained for F-actin (ActinRed 555) and nucleus fluorescence (PureBlu Hoechst 33342) as well as SA-β-galactosidase activity (CellEvent Senescence Green). Positive control for senescence were HCT116 cells incubated with 50nm Doxorubcin for 72h. Results were obtained by fluorescence

microscopy and flowing cytometry, then processed with Flowing Software, Leica LasX and ImageJ.

Results

Microscopic images analysis presented bigger average nucleus area after decitabine exposure. Cytofluorimetric fluorescence intensity measurement of HCT116 cells showed higher F-actin expression even 7 days after treatment and indicated SA-β-galactosidase activity. Observed changes were time-dependent and correlating with worsening cells condition.

Discussion and conclusions

Those results show that induced by decitabine morphological changes were reflected by increased F-actin quantity and bigger nucleus area which is likely a manifestation of drug-induced senescence.

Acknowledgements

This research was supported with grant OPUS 2017/25/B/NZ5/02608 and statute programme IITD 3/2019.

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Influence of manufacturing parameters on alginate-gelatin hydrogels for 3D cell culture

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Background

Hydrogels, as cross-linked polymeric networks, by the content of hydrophilic groups are materials able to bind large amounts of water. They have been present in medical applications since the 1960s and nowadays are suitable for pharmaceutical applications [1]. Hydrogel *in vitro* cell culture media are relevant to sustain cells and create an environment similar to the *in vivo* conditions due to their high permeability to oxygen, nutrients or other water-soluble compounds, furthermore, they allow cells to migrate freely in any direction. For this purpose, either natural (e.g., alginate, chitosan, collagen, fibrin, gelatin) and synthetic polymers are used. Natural polymers are widely used for their similarity of mechanical properties to human tissues and their biodegradability. Unfortunately, their durability is limited and their composition may be variable [2, 3].

There are technologies available for culturing cells in 3D such as hanging drop method, low-binding plastic, pyramid plates, rotary cell culture, scaffold based cultures etc.. Technologies of alginate hydrogels include beads, delayed gelation systems, macroporous scaffolds, honeycomb scaffolds with pore structure, scaffolds with nanoparticles and 3D printed scaffolds. Stem cell differentiation and alginate hydrogels elasticity matching to most types of tissue can be controlled by optimizing type of alginate, its concentration and selection of cross-linking technology. Bioprinting may use materials (e.g., alginate hydrogel, as a bioink) and cells, they should be biocompatible, to form a variety of 3D formats where cell function and viability are preserved within the printed structure [3, 4].

The aim of present paper is evaluation of parameters manufacturing and material influence on maintenance culture of tumor cells from the line MCF-7/DOX breast cancer cells and describe fabrication of alginate-gelatin hydrogel media for the cell culture, as well as assessment of mechanical properties of cross-linked hydrogel. Alginates are naturally occurring anionic polymers obtained from brown seaweed, able to form a gel in the presence of bivalent ions, e.g. Ca^{2+}. They are used often due to their rheological properties, biocompatibility, as well as lack of toxicity. Sodium citrate chelates calcium ions and is used to dissolved cross-linked gels. Changed hydrogel by sodium citrate can be more suitable environment for cells – can be printed and retain their capacity to proliferate and group [1, 5].

Material and Methods

In this paper, a line of MCF-7/DOX tumour cells has been used for the investigation. Hydrogel cell culture media were created using physiological buffered saline (PBS) with 5% alginate and 20% gelatine composition. The hydrogel cross-linking was carried out chemically with calcium ions, in $CaCl_2$ solutions. Sodium citrate was added for controlled dissolved alginate gel [5].

Cell formation was observed on different configurations of alginate hydrogel substrates: depending on the presence of sodium citrate as well as the structure of cross-linked hydrogel created by the appropriate amount of calcium ions.

Results

In this study has been achieved optimal protocol to obtain alginate-gelatin hydrogel which is sterile and non-toxic for cell culture. It has been also conducted evaluation of sodium citrate on viability of MCF-7/DOX cells.

Discussion and conclusions

In this work has been realized the initial optimization of alginate-gelatin hydrogel obtaining as a bioink for 3D printing. Cross-linking of the hydrogel has an effect on obtaining desired mechanical properties by selection of appropriate CaCl2 solution concentrations, which in consequence influences the cell culture growth.

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Metabolic differences between subcutaneous and visceral adipose tissues based on gene expression study

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Background

Adipose tissue is the main energy reservoir in the body. Besides the energy storage, it plays the role of endocrine organ secreting numerous biologically active peptides called adipokines. Pathological and excessive accumulation of adipose tissue, both visceral (VAT) and subcutaneous (SAT), is influenced by metabolic, psychological, endocrine and genetic factors [1]. Many scientific reports indicate a high correlation between obesity and the aberrant gene expression profile [2]. Although a greater impact on the development of metabolic disorders is attributed to VAT, it turns out that SAT may play an equally important role in this process [3]. Therefore, we have attempted to compare the metabolism activity of both types of adipose tissue. For this purpose, we have analyzed in VAT and SAT the group of 27 genes associated with the insulin pathway, adipokines, cytokines, lipids, and transcription factors regulating the development and metabolism of adipocytes, and transcription factors regulating cell responses to hypoxia.

Material and Methods

Visceral and subcutaneous adipose tissue biopsies were collected during abdominal surgeries from 18 patients in the BMI range from 20 to 27, in the age from 40 to 60 years, with HOMA-IR < 2,5 and with equal distribution of sex. The total RNA was isolated using a combination of trizol method and commercial column spin method kits. Reverse transcription was performed with the use of High Capacity cDNA Reverse Transcription Kit. Gene expression was done using Real-Time PCR based on SYBR Green assay. A relative gene expression level, normalized to the housekeeping gene (β-actin), was calculated using the delta-delta Ct (ΔΔCt) model.

Results

A comparative analysis showed strong statistical significant differences in the expression between VAT and SAT occur in the case of *LEP* (*Leptin*) (p = 0,022) and *IGF2* (*Insulin like growth factor 2*) (p = 0,002). It was observed that the expression of *LEP* was about twice lower in VAT than in SAT, but in case of *IGF2* was about twice higher in VAT than in SAT. Moreover, in visceral adipose tissue, we observed also significantly increased expression level of *Il-10* (*Interleukin 10*), *PIK3R1* (Phosphoinositide-3-kinase regulatory subunit 1), *CEBPβ* (*Enhancer binding protein beta*), *TNFα* (*Tumour necrosis factor alpha-like*) and *PPARGC1A* (*PPARG coactivator 1 alpha*), and significantly decreased expression level of *SLC2A4* (*Solute carrier family 2 member 4*), *SCD1* (*Stearoyl-CoA desaturase*) and *Il-6* (*Interleukin 6*) compare to subcutaneous adipose tissue, but these results didn't show a statistical significance.

Discussion and conclusions

The obtained results indicate metabolic differences between VAT and SAT. Subcutaneous adipose tissue is seems to be much more involved in the process of lipogenesis, which is indicated by increased expression of genes associated with lipid metabolism, especially in fatty acids synthesis (*SCD1*) and also by increased expression of gene encoding a leptin, which is considered an energy sensor that regulates appetite and the amount of adipose tissue in the body. While the higher expression level of *LEP* is probably the result of the body's response to increased lipid production. In turn, visceral adipose tissue shows increased susceptibility to inflammation, which is indicated by increased expression of genes encoding inflammatory factors. However, the reduced expression of *SLC2A4* in VAT with the simultaneously significant increased expression of *IGF2* and also higher

expression of *PIK3R1* compere to SAT may indicate a greater risk of disturbances in the insulin pathway, which may lead to the development of insulin resistance in adipocytes in this type of adipose tissue.

Financial support:
The study is supported by The National Science Centre (number of the research project: 2016/21/D/NZ5/00155). The research protocols and all procedures were approved by the Ethical Review Board of Wroclaw Medical University. Approval No. KB-124/2017.

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Long-term treatment with indomethacin increases the number of PACAP-immunoreactive porcine duodenal neurons

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Background

Due to numerous therapeutic applications and high availability, non-steroidal anti-inflammatory drugs (NSAIDs) are the most widely used drugs worldwide [1]. Enteric neurons are characterized by considerable chemical plasticity and the appearance of a pathological factor results in a change in the synthesis of neurotransmitters [2;3]. Therefore the aim of the study was to determine the effect of inflammation caused by indomethacin supplementation on pituitary adenylate cyclase-activating peptide (PACAP) expression in enteric duodenal neurons in domestic pigs.

Material and Methods

The study was carried out on eight imamture pigs of the Pietrain x Duroc race (approximately 20 kg of body weight, about 8 weeks old). The animals were divided into two groups – a control (C group) and an experimental group (I group). Group C (n=4) was consisted of animals which received empty gelatine capsules. Group I (n=4) was composed of pigs which indomethacin (10 mg/kg b. w.) were given orally for 4 weeks, approximately 1 h before feeding. After this time, animals from both groups were euthanized. Then, frozen sections (14 μm thickness) were prepared from the collected material (3 cm fragments of duodenum located 10 cm caudal to musculus sphincter pylori) and subjected to double immunofluorescence staining. Antibodies against the neuronal marker PGP 9.5 and against the pituitary adenylate cyclase-activating peptide were used as primary antibodies. The secondary antibodies – Alexa Fluor 488 and 546 – were also used for staining. Analysis of the sections was performed using an Olympus BX51 fluorescence microscope.

Results
Microscopic analysis showed significant increase in the number of PACAP positive neurons, both in the myenteric and submucous plexuses of the porcine duodenum.

Discussion and conclusions
Increased number of the PACAP-immunoreactive neurons in the myenteric and submucous plexuses following indomethacin evoked duodenal inflammation may reflect down regulation of the inflammatory process. The results show that indomethacin, through inhibition of cyclooxygenase and thus prostaglandins synthesis, impairs the mucus/bicarbonate duodenal barrier. To restore intestinal homeostasis and counteract inflammation, local enteric neurons are subject to a chemical adaptation process. To synthesis and release an additional volume of protective neurotransmitters such as PACAP, the ENS recruits additional neurons, thus increasing the number of operating cells [2]. Since the chemical plasticity of the enteric neurons constitutes the basis of gastrointestinal compensatory mechanisms, the presented results may contribute to the future development of new strategies for the treatment of gastrointestinal diseases

This study was supported by the National Science Centre (grant no. 2018/29/N/NZ4/00348).

*Tab. 1 Percentages of immunoreactive enteric neurons in the porcine duodenum in the control and indomethacin-treated animals; **p < 0.01,*

Active neuronal substance	Type of plexus	Control group	Indomethacin group
PACAP	MP	9.69 ± 0.37%	11.91% ± 0.13% **
	OSP	10.51 ± 0.31%	14.02% ± 0.21% ***
	ISP	12.82 ± 0.35%	16.36% ± 0.26% ***

****p < 0.001 indicate differences in PACAP expression in comparisons to the control animals.*

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The relationship between TNF-α, CDKN1A/p21 and MMP9 in esophageal squamous cell carcinoma

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Background
Esophageal squamous cell carcinoma (ESCC), a highly aggressive and often late diagnosed disease, is mostly induced by chronic inflammation [1]. Inflammation generally plays crucial role in tumorigenesis, tending to promote cancer invasion and metastasis, the latter being clinically the most critical aspect of the disease [2]. Thus, most elements of the machinery involved in inflammation-driven cancer progression may work as a target for therapy. In the

multistep process of metastasis, remodelling of extracellular matrix (ECM) allows cancer cells to invade and migrate through their microenvironment. Matrix metalloproteinases (MMPs) are crucial for the ECM degradation and they determine the aggressiveness of malignant cells. Gelatinase MMP-9 was found to be commonly upregulated in human cancers, and besides cleaving type IV collagen, laminin and elastin, it affects cell signalling by processing chemokines, growth factors or cell receptors [3; 4]. This enzyme is also a major gene controlled by transcription factor NFκB, which states the link between metastasis and TNF-α, an inflammation cytokine and mentioned signalling pathway activator. Recently it was shown that cyclin-dependent kinase inhibitor (CDKN1A/p21) plays an important regulatory role in TNF-α-induced MMP9 gene expression in triple-negative breast cancer [5]. CDKN1A/p21, apart from arresting cell cycle, can protect the cell from apoptosis, yet its actual role as either tumour suppressor or an oncogenic factor seems to be environment-dependent.

Material and Methods

In this study, using real-time PCR and immunohistochemistry (IHC) we investigated levels of CDKN1A/p21, MMP9 and TNF-α expression on both mRNA and protein levels in ESCC tissues, and the relationship between CDKN1A/p21 and MMP9 in human squamous cancer cells of the esophagus, KYSE70, by transfecting cells with CDKN1A/p21 siRNA and treating them with TNF-α.

Results

Using real-time PCR, we found that the expression of CDKN1A/p21 and MMP9 as well as TNF-α genes was significantly increased in cancer tissues compared to the control groups. These results were confirmed by IHC. We also found that the TNF-α treatment of human esophageal squamous cancer cells, in *in vitro* conditions, resulted in the statistically significant increase in expression levels of both CDKN1A/p21 and MMP9. Next, using gelatin zymography, we observed that siRNA-induced transcriptional silencing of CDKN1A/p21 gene inhibited TNF-α-dependent MMP9 expression.

Conclusions

CDKN1A/p21 may play an important role in the development of ESCCs by its contribution to the regulation of TNF-α-induced MMP9 expression.

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Singlet oxygen photogeneration by biological staining dyes

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Background
Photodynamic therapy, synthesis of fine chemicals or wastewater treatment, these are few of the most important uses of singlet oxygen ($^1O_2$) photogeneration [1]. The significance of singlet oxygen in chemistry, biology or medicine is highly appreciated by researchers around the world. It is often formed in photosensitization processes where photoactive compound and source of light of appropriate wavelength are required [2]. Considering great photoactive properties, biological dyes have variety of applications thus they can be used as a photosensitizers. In this work compounds such as tropaeolin, rose bengal and crystal violet were tested for their photoactive properties.

Material and Methods
Tropaeolin, rose bengal and crystal violet solutions in methanol (Sigma Aldrich) were used in singlet oxygen photogeneration. Measurements were done using UV-Vis spectroscopy in quartz cuvettes by investigating homogeneous mixtures of given compound with specific chemical trap – 1,3-diphenylisobenzofurane (DPBF, Sigma Aldrich). Source of light was lasers working at wavelength 532 nm.

Results
UV-Vis measurements were based on tracking in decrease absorbance at wavelength appropriate for chemical trap (410 nm). Total decreases of absorbance stand at 0.59, 1.06 and 0.97 for tropaeolin (from 1.07 to 0.48), rose bengal (from 1.15 to 0.09) and crystal violet (from 1.11 to 0.14) respectively. Measurements total time in each case was 240 seconds.

Discussion and conclusions
Photogeneration of $^1O_2$ occurs when light-activated molecules of photosensitizer are causing excitation of oxygen molecule from its ground state. Chemical traps, such as DPBF are used to indicate this process by observing decrease of absorbance, mentioned in results. That indeed is an outcome of the DPBF reaction with oxygen molecules which results in its oxidation.

All given compounds have shown relatively good ability to singlet oxygen generation in homogeneous systems. This gives opening to preparation of heterogeneous $^1O_2$ photogeneration systems in which photosensitizers can be deposited on solid surfaces.

This work was supported by European Social Fund in the framework of the project "Silesian University of Technology as a Center of Modern Education based on research and innovation" POWR.03.05.00-00-Z098/17.

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Long-term melatonin treatment accelerates myocardial activation processes

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Background
Melatonin is thought to have antiarrhythmic properties in ischemia/reperfusion conditions. Previous studies from our group demonstrated that the antiarrhythmic effect of melatonin was associated with improved ventricular activation, but exact mechanisms are unclear. The improvement of activation may be caused by enhancement of propagation via His-Purkinje system and/or intramyocardial conduction. The present study aimed to assess effects of long-term melatonin treatment on epicardial activation time (AT) and conduction velocity (CV) in rat hearts.

Material and Methods
Experiments were performed in a total of 44 anesthetized open-chest male Wistar rats. The animals received melatonin (10 mg/kg/day, single oral dose) or placebo for seven days. Unipolar electrograms were recorded from the epicardium of the right ventricle (RV) and left ventricle (LV) using an array of 64-leads. In each lead, AT was determined as an instant of dV/dt min during QRS complex, and isochronic activation maps were constructed. CV was measured during electrical stimulation (400 bpm, 2 mA, 2 ms) in the middle of the LV and RV free walls. Anisotropy of conduction was estimated as a ratio between a longitudinal (CV max) and transversal (CV min) CVs.

Results
Melatonin reduced ATs in the LV (11.14±0.96 vs 13.1±0.71 ms, $p<0.001$) and RV (10.8±0.78 vs 11.71±0.99 ms, $p=0.010$) in the melatonin (n=13) and control (n=12) groups, respectively. In the LV, CV demonstrated marked anisotropy. CV max (0.74±0.17 vs 0.73±0.13 m/s), CV min (0.43±0.12 m/s vs 0.36±0.10 m/s) and CV max/CV min (1.8±0.5 vs 2.1±0.7) did not differ significantly in the melatonin-treated (n=10) and untreated (n=8) animals, respectively. However, the RV CV being isotropic was higher in the melatonin-treated animals as compared to controls (0.66±0.1 vs 0.49±0.1 m/s, $p=0.027$, respectively).

Discussion and conclusions
Long-term melatonin treatment led to myocardial activation enhancement, which was at least partly due intramyocardial conduction acceleration in the RV. On the other hand, activation time shortening by melatonin in the LV implies involvement of conduction system.

The study was supported by Russian Science Foundation (RSF 18-15-00309).

The antimicrobial effectiveness of bacterial cellulose dressings chemisorbed with commonly used wounds irrigation agents against chosen opportunistic pathogens

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Background
Bacterial bionanocellulose (BC) is a biomaterial produced by bacteria *Komagataeibacter xylinus*. BC has a number of properties which make it an excellent biomaterial for medical applications. The previous research indicated that BC facilitates the

proper healing process by the maintenance of optimal hydration of wounds. The high water-related properties of bacterial cellulose allows to enrich the dressing with various substances, including antimicrobial agents [1,2]. Topical antibiotic therapy is not recommended to treat wound infections. The reason is poor penetration of antibiotics into the wound and a high risk of selecting antibiotic-resistant strains. For wound irrigation, sterile antiseptics or lavaseptics are recommended. The octenidine dihydrochloride (OCT), polyhexamethylene biguanide (PHMB) and super-oxidized solutions of hypochlorites (NaOCl) are examples of such liquids containing also antimicrobial activity [3].

The aim of this research was the evaluation of antimicrobial effectiveness of bacterial cellulose dressings chemisorbed with commonly used wounds irrigation agents against chosen opportunistic pathogens.

Material and Methods

Material: Research was carried with use of 4 reference strains of bacteria: *Staphylococcus aureus* ATCC 6538, *Staphylococcus aureus* ATCC 33591, *Klebsiella pneumoniae* ATCC 4352 and *Pseudomonas aeruginosa* ATCC 15442 and 2 clinical strains of each examined species. Bacterial cellulose was produced by *Komagataeibacter xylinus* ATCC 53524. All strains come from the collection of the Department of Pharmaceutical Microbiology and Parasitology. The tested antimicrobial agents were: octenidine (Octenilin®, Shülke), polyhexanid (Prontosan®, B.Braun) and super-oxidized solution of hypochlorites (Microdacyn®, Kikgel).

Methods: To evaluate antimicrobial action of tested compounds the minimal inhibitory concentration (MIC) and minimal biofilm eradication concentration (MBEC) tests were carried out. To evaluate antibacterial action of BC dressings saturated with tested compounds the modified disc diffusion method was performed.

Results

In the MIC test the strongest action of PHMB and OCT on staphylococci and the weakest against *P. aeruginosa* was observed. NaOCl did not show any bactericidal activity in the tested concentration range. Under the experimental conditions, PHMB had the strongest effect on all strains except *P. aeruginosa*, for which OCT was better.

Tested agents acted much weaker on the biofilm than on the planktonic forms. OCT was most effective against biofilm created by staphylococci, PHMB on biofilm created by *K. pneumoniae*, while NaOCl did not show the biofilm eradication ability at all. None of the tested agents had an activity against the biofilm formed *by P. aeruginosa*.

In the modified diffusion-disc method, in which BC chemisorbed with analysed compounds was applied, PHMB and OCT were effective against all tested bacterial strains, while no growth inhibition zone around the BC disc chemisorbed with NaOCl solution was observed. PHMB turned out to be the most active compound in the experimental conditions.

Conclusions

Bacterial cellulose is a suitable material for dressings of antibacterial activity. Antimicrobial agents are released from the BC without the loss of effectivity. The active substances dilution in BC does not significantly affect activity of PHMB and OCT.

PHMB and OCT have widely proven antimicrobial activity, which is confirmed by the presented research.

According to data provided here, NaOCl did not show any bactericidal activity, even at the highest concentration (undiluted). Our results are contrary to other scientific reports concerning matter discussed. These

discrepancies may be result of differences in testing models used by our vs. other teams. More research is needed to draw final conclusions on the antibacterial activity of NaOCl [4,5].

Research was performed by means of statutory funding SUB.D 230.20.002 and funding for young researchers STM.D230.20.053.

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Hutchinson-Gilford Progeria Syndrome therapy with RNA interference approach

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Background

Hutchinson-Gilford progeria syndrome (HGPS) is an extremely rare genetic disorder caused by a point mutation in the *LMNA* gene (C1824T). This gene codes for lamin A and C proteins, however, the mutation localizes in lamin A coding sequence. Both, lamin A and C are structural proteins of the nuclear envelope, which provide mechanical support to the nucleus.

De novo point mutation in exon 11 of lamin A rises new splice site in mRNA. Thereby mature mRNA lacks part of the coding sequence. Such mRNA codes for the shorter version of lamin A called "progerin".

After the synthesis in the cytoplasm, lamin A C-terminus is farnesylated. This post-translational modification enables transport into the nucleus, where anchors protein to the inner nuclear membrane. At the next step of the maturation, farnesylated C-terminus is cleaved by endoprotease, it releases a mature form of lamin A.

The progerin lacks signaling sequence recognized by endoprotease. For this reason, it accumulates into the nucleus remaining attached to the nuclear membrane. This, in turn, results in the progeroid cell phenotype – nuclear envelope disruption, loss of peripheral heterochromatin and abnormal gene signaling.

Nowadays, effective treatment for HGPS doesn't exist. The therapy is limited to moderation of the symptoms such as atherosclerosis, prevention of stroke and myocardial infarction. Preclinical therapies are focused mainly on progerin levels reduction using autophagy induction, farnesylation inhibition or aberrant splicing downregulation.

In our study, we were aimed to develop the therapy which would decrease the synthesis and accumulation of progerin in the nuclear envelope. We used the RNA interference approach to specifically downregulate progerin expression. The set of siRNAs

sequences was designed to recognize the junction between exon 11 and 12 in progerin mRNA, but not lamin A. One sequence was tested in combination with a clinically approved drug for HGPS therapy – lonafarnib.

Material and Methods

HeLa cells were transduced with retroviruses to overexpress GFP, GFP-lamin A or GFP-progerin. Transduced sublines were next transfected with designed siRNAs. The efficiency of siRNA to downregulate GFP-progerin level was indicated by the measurement of fluorescence intensity with flow cytometry. Results were confirmed with western blotting analysis and fluorescence microscopy.

One of the selected siRNAs sequences was tested in combination with lonafarnib and analysed with the same methods.

Results

Designed HGPS cellular model based on HeLa cells was an effective tool to fast and easy siRNAs sequences screening. Two aspects were taken into the account during the selection – siRNA efficiency and specificity.

Designed siRNAs were able to reduce the level of GFP-progerin up to 25% as flow cytometry results showed. Obtained results were confirmed by western blotting analysis and fluorescent microscopy.

We investigated the effect of a combination of siRNA and lonafarnib treatment, as expected no antagonistic effect was observed.

Discussion and conclusions

The accumulation of progerin in the nuclear envelope cause defects in nuclear envelope structure and functions, thus the reduction of progerin level seems to be crucial for effective progeria treatment.

Our results shows the sufficient decrease of progerin level in transduced HeLa cell line without affecting the lamin A level. Besides, an additive effect of the combination of siRNA and lonafarnib treatment lets us consider combined therapy for further study with patients fibroblasts cells.

Among different preclinical HGPS treatment strategies, Huang et al. study was based on RNA interference approach with shRNAs sequences. One of shRNAs sequences was shown to specifically decreases the progerin level in immortalized patients fibroblasts. However, in our cellular model, this sequence showed weak efficiency.

The research is supported by grant ERA-NET-E-RARE-3/III/TREATHGPS/10/2018 from the Polish Agency NCBR.

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Little fish, big case: zebrafish as a model of human metabolic disease

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Background

The zebrafish (*Danio rerio*), small aquarium fish, is a model organism used in research concerned on human diseases, since zebrafish genome shows high similarity with humans [1]. One of such example is the case of human metabolic McArdle disease caused by muscle form of glycogen phosphorylase (PYGM) deficiency. PYGM is an enzyme, which attend in the spread of glycogen in the first step of glycogenolysis. Mutation in the *PYGM* gene leads to autosomal recessive McArdle disease in humans. Patients suffer from muscle aches, cramps and fatigue during physical exercise. Such symptoms occur due to lack of available glucose in the muscles. So far, no efficient treatment has been found.

The aim of our research was to determine if zebrafish could be an animal model for human McArdle's disease.

Material and Methods

To get a better look at the role of muscle glycogen phosphorylase in zebrafish we knockdown the pygm using a morpholino technique. We also use behavioural tests and statistical analysis.

Results

Our previous observations indicated that *pygm* gene knockdown, performed with morpholino oligonucleotides, leads to morphological changes mimicking the symptoms of McArdle disease [2].

Here we show the effect of Pygm knockdown on zebrafish physical performance. The results of behavioural assay shows that indeed the Pygm deficiency in zebrafish decreases its motility.

Discussion and conclusions

Our results confirmed our previous assumption that zebrafish could be a good model organism to investigate the human McArdle's disease.

Acknowledgement:

This work was *supported* by National Science Centre (grant no. 2017/01/X/NZ4/00093) and the Polish State Committee for Scientific Research, Project No. 1068/S/IBE/2018.

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Effect of electroporation on the immunogenicity of murine Lewis lung carcinoma cells

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Background

Tumor cells have developed various mechanisms that allow them to escape from immune surveillance. One of them is that tumor cells are poorly recognized by immune cells.

The aim of the study was to evaluate if electroporation of cancer cells entails changes in tumor immunogenicity.

Material and Methods
Murine Lewis lung carcinoma (LLC) cells were electroporated using nano- or microsecond pulsed electric field. The effectiveness of electroporation, defined by cell membrane permeabilization efficacy, was evaluated using propidium iodide uptake assay performed shortly after the electroporation.

To determine changes in immunogenicity of LLC cells after electroporation, the MHC class I expression on the surface of electroporated LLC cells was examined using flow cytometry.

Moreover, their influence on the differentiation of dendritic cells and indirectly on the activation of specific antitumor response was further determined.

Results
Selected electroporation parameters caused effective permeabilization of LLC cell membranes, without induction of cell death. It was observed that some of selected electroporation parameters, especially microsecond electric pulses, induced increased expression of MHC class I on the surface of LLC cells. Moreover, dendritic cells cultured in the presence of electroporated cells were characterized by higher expression of MHC class II and costimulatory antigens than dendritic cells cultured in the presence of control LLC cells. Mature dendritic cells were more effective in the activation of anti-LLC immune response.

Discussion and conclusions
Certain electroporation conditions induced an increase in immunogenicity of LLC cells thereby improving their recognition by dendritic cells. Based on the obtained results we conclude that electroporation could have an application in the preparation of DC-based anticancer vaccines.

This study was funded by National Science Centre, Poland (project no 2018/30/E/NZ5/00711).

Polymers as the catalysts for nucleation and growth stable and metastable cocrystal polymorphs

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Background
Pharmaceutical cocrystals are the subject of interest in academic and industrial research as they offer better control over physicochemical, mechanical and pharmacokinetic properties of active pharmaceutical ingredient (API) while its therapeutic activity remains intact. This class of materials, as well as single component pharmaceutical solids, are prone to exhibit the different packing arrangements and molecular conformations within the crystal lattice with the same chemical composition (polymorphism).

In this work we use polymer assisted grinding (POLAG) as a mechanochemical method to control nucleation and growth of stable and metastable cocrystal polymorphs[1].

Material and Methods
Two cocrystals known to exist in at least two polymorphic forms were selected: (1) theophylline (TP) with benzamide (BZ) and (2) nicotinamide (NCT) with malonic acid

(MA). The milling experiments were performed using ball-grinder (FRITSCH Mini-Mill PULVERISETTE 23). Excipients used as cocrystallisation catalysts: PEG (of varying molecular weight), SPAN 80, TWEEN 20, TWEEN 80, 1,2-penthanediol, propylene glycol, Brij® 93, Pluronic® L-35 and Pluronic® L-31.

Structure of obtained cocrystals were investtigated using X-ray powder diffraction (PXRD) and Fourier Transform Infrared Spectroscopy (FTIR). Thermal transitions of cocrystals and physical mixtures of APIs, coformers and excipients were assessed using differential scanning calorimetry (DSC).

Results

Both polymorphic forms of TP:BZ (1:1) cocrystal were obtained by neat and liquid assisted grinding (NG and LAG) as reported previously[2]. Mechanochemical synthesis of a TP:BZ cocrystal using all tested excipients resulted in formation of polymorph I.

In the other investigated system, NCT:MA (2:1), all grinding procedures produced exclusively the NCT:MA form I, with no trace of form II[3].

The DSC analysis of selected excipient mixtures with APIs, and coformers enabled us to better understand the effect of polymer addition on the cocrystallisation process.

Discussion and conclusions

Polymorphic screening of a given compound (cocrystal) is an important and integral step of new drug form development. Complete knowledge of solid-state properties enables to make a decision on most suitable polymorph that should be used in further development to prevent unwanted structural changes during the formulation and storage of the final product.

POLAG method could be applied as a method of screening for polymorphic forms of cocrystals. Moreover, used in low quantities polymers could possibly act as cocrystallization rate accelerating agents. The use of pharmaceutical excipients with desired technological properties may enable to control the properties of a final product e.g. drug release, tabletting properties, hydrophilicity.

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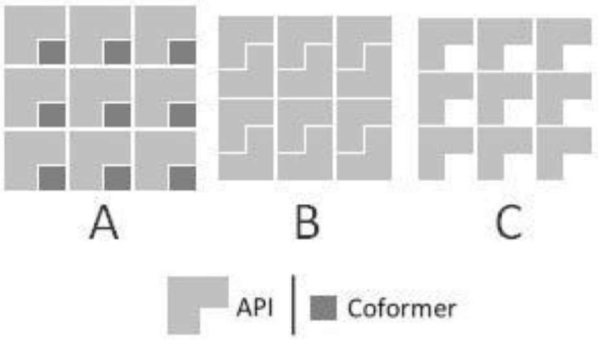

Fig.1. Schematic representation of (A) a cocrystal; (B) and (C) API polymorphs.

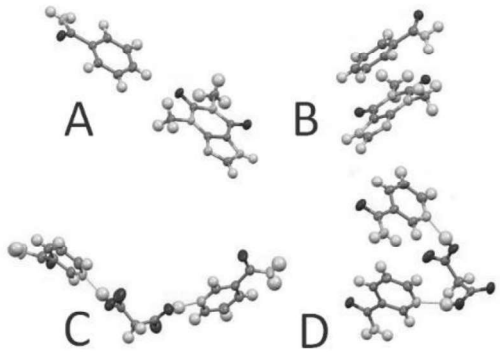

Fig.2. Molecular motfis of the (A)TP:BZ form I; (B)TP:BZ form II; (C)NCT:MA form I; (D)NCT:MA form II

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Escitalopram affects hypocretins/orexins transmission in hypothalamus of stressed rats

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Background

Growing evidence from pre- and clinical studies suggests that hypocretins/orexins transmission plays a role in the patomechanism of psychiatric disorders (e.g. anxiety and depression). Furthermore, this transmission is involved in mechanism of action of SSRIs (e.g. escitalopram). The main mechanism of action of escitalopram consists of 5-HTT inhibition and regulation of HPA axis activity. It is known that this drug alters neurotrophic factors action in the limbic system. Further, may affect some neuromodulators (e.g. galanin, oxytocin, vasopressin and corticotropin releasing factor).

Material and Methods

Maternal separation (MS) was used as a model of depression and anxiety. Pups of Wistar rats were maternally separated from 2-15P for 6h per day (between 9am and 3pm). In the adulthood (2,5 months) males from stressed or control groups were assignned to saline or escitalopram exposure. Drugs (10mg/kg ip) or saline were administrated once daily for 21 days. The rats were sacrificed 24h after the last dose of drug. Dissected brains were homogenized in TriZol. Orexins system was evaluated by RT-qPCR method.

Statistical analysis was evaluated by ΔCt method and t-student test. All the estimations performed using GraphPad 7.04.

Results

OX-A mRNA relative expression

In control + escitalopram group relative expression factor indicated [R]=4; in stressed rats [R]=5 and in stressed + escitalopram [R]=4.

OX1R mRNA relative expression

In statistical significant comparison was observed [R]=4 in control + escitalopram group; in stressed rats [R]=3,5

Discussion and conclusions

Early-life stress induced up-regulation of orexin-A expression level in the hypothalamus. Chronic treatment with escitalopram did not alter orexin-A expression level in the stressed rats. Orexin receptor 1 expression level slightly enhanced in the stressed rats. Interestingly, both orexin-A and orexin receptor 1 expression level increased in the non-stressed rats. The activity of hypocretins/orexins transmission might be modulated by stress and escitalopram.

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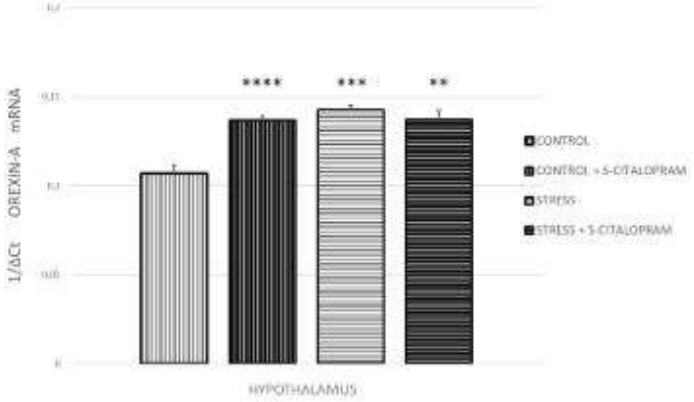
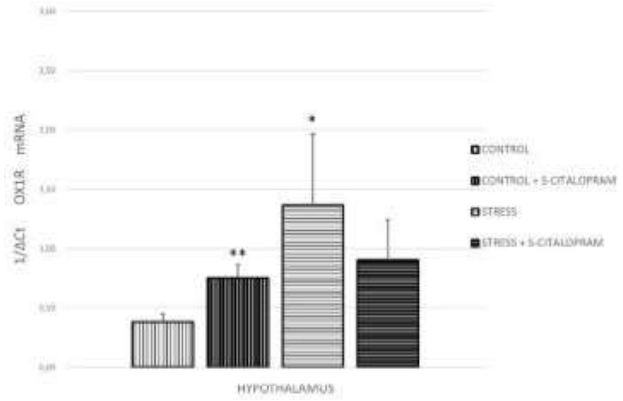


***p<0.01; ***p<0.0005; ****p<0.0001 two-tailed t-student*



**p<0.05; **p<0.001 two-tailed t-student*

Downregulation by IL-33 of Map/Erk signalling pathway in gastric epithelial cells in response to *H. pylori*, as a potential mechanism of controlling inflammatory response

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Background

Infection with *H. pylori* Gram-negative bacteria causes gastritis or gastric ulcer in humans results in the gastric barrier damage. Interleukin (IL)-33 is a proinflammatory cytokine that alerts the host immune system in response to a homeostasis disorder.

Aim

In this study we asked whether IL-33 is upregulated in gastric barrier cells exposed to *H. pylori* components, and whether controls the Map/Erk (mitogen-activated protein kinases/extracellular signal-regulated kinases) signaling pathway.

Material and Methods

Primary gastric epithelial cells and fibroblasts of *Caviae porcellus* sensitive to *H. pylori* infection, nontransfected or transfected with IL-33 siRNA, were exposed in the cells cultures *in vitro* to *H. pylori* antigenic complex glycine acid extract (GE). The level of IL-33 before/after siRNA IL-33 tranfection of cell was measured the cell culture supernatants by the ELISA test (MyBiosource). Furthermore, cells were stained with anti-IL33 antibody, FITC conjugated (ThermoScientific) and imaged in the confocal microscope (Leica SPE). Similarly, activation of Erk was evaluated by staining the cells with FITC conjugated antibodies to phosphorylated Erk (Cell Signaling). The fluorescence intensity was measured using the Victor 2 reader (Wallac) at the wavelengths 495 nm (excitation) and 519 nm (emission).

Results

Primary gastric epithelial cells and fibroblasts non-transected with IL-33 siRNA when treated with *H. pylori* GE produced significantly increased amount of IL-33 as compared to control cells. By comparison cells tranfected IL-33 siRNA control or GE-induced, were not able to produce IL-33. The level of phosphorylated Erk in IL-33 siRNA-silenced cells, treated with GE was significantly higher than in nontransfected cells. These results indicate that IL-33 controls the activation of Map/Erk signaling pathway.

Discussion and conclusions

Down regulation by IL-33 of *H. pylori*-induced Erk activation in gastric tissue cells may be an important mechanism protecting the gastric barrier of the host from loss of homeostasis and the development of excessive inflammatory response related to the infection.

Downregulation of MUC5AC production by BCG-onko mycobacteria in *in vivo* model of *Helicobacter pylori* infection

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Background

Helicobacter pylori is an etiological agent of chronic gastritis, duodenal ulcers and gastric cancer. Colonization of gastric epithelial cells is mediated by *H. pylori* adhesins with host mucins, including mucin 5 (MUC5AC). The *M. bovis* mycobacteria present in BCG-onko vaccine are known to posses the immunomodulatory activity. This vaccine is widely used for immunotherapy of bladder cancer. Due to increasing antibiotic resistance of *H. pylori*, alternative methods of elimination of these infection are being considered. We asked whether BCG-onko vaccine mycobacteria given to guinea pigs sensitive to *H. pylori* infection are able to modulate MUC5AC production in gastric tissue, which potentially may diminish *H.pylori* colonization

Material and Methods

Himalayan *Cavia porcellus* male or female (500-800g) were used, as a model of *H.pylori* infection. Animals were bred in the Animal House at the Faculty of Biology and Environmental Protection, University of Lodz (Poland), kept in cages with free access to drinking water and fed with standard chow. Experiments were approved by the Local Ethics Committee LKE9 (Decision 58/ŁB45/2016).

Animals were inoculated *per os* with *H.pylori* CCUG17874 reference strain (10^{10} CFU/ml) in *Brucella* broth, 3x at 2 days intervals. Before or after administration of *H.pylori* the animals received BCG-onko mycobacteria (1×10^{8} CFU/ml) (Biomed, Lublin, Poland) by the oral route. Control animals were inoculated only with *Brucella* broth or BCG-onko. The guinea pigs were euthanized after 7 or 28 days from last inoculation. *H.pylori* infection was confirmed by histological staining (hematoxylin-eosin, Giemsa), and molecular (PCR for *cagA /ureC* genes) examination of gastric tissue as well as the production of anti-*H.pylori* antibodies. In parallel the inflammatory response was assessed.

Deposition of MUC5AC in gastric tissue was evaluated using anti-MUC5AC antibody (MyBiosource, USA) and FITC conjugated secondary antibody.

Results

In *H. pylori* infected animals the production of MUC5AC was significantly increased, after 7 and 28 days from inoculation, as compared to non infected animals. BCG-onko mycobacteria, which were given to animals alone or in combination with *H.pylori,* significantly diminished the MUC5AC production, which was correlated with downregulation of gastric tissue colonization with *H.pylori* (previous study[1]).

Discussion and conclusions

BCG-onko mycobacteria by diminishing MUC5AC production in the gastric mucosa of *Cavia porcellus* were able to prevent colonization of *H.pylori*. Further study is necessary in order to elucidate the role of BCG-onko mycobacteria in modulation of MUC5AC production.

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Autoantibodies crossreacting with TNFR, induced in response to *H.pylori* CagA protein during experimental infection in *Cavia porcellus*

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Background

Helicobacter pylori are Gram-negative rodes, colonizing gastric epithelial cells in humans which may induce crossreacting antibodies and adverse inflammatory reaction due to the antigenic mimicry between bacterial and host components. Bioinformatics analysis was used to show the similarity between *H.pylori* cytotoxin-associated gene A (CagA) protein and human as well as guinea pig tumor necrosis factor receptor (TNFR). *Cavia porcellus*, which are susceptible to *H.pylori* infection were used to study the induction of anti-TNFR cross-reactive antibodies in *Cavia porcellus* in response to inoculation with *H.pylori* CagA positive strain.

Material and Methods

Serum samples were collected from control (10) or *H.pylori* infected animals after 7, 28 and 60 days (10,20,10) from inoculation. The induction of IgM and IgG antibodies towards *H.pylori* antigens was determined by laboratory ELISA with the glycine acid extract (GE) – complex of surface antigens or with recombinant CagA protein (IRIS, Siena, Italy) from the reference strain. The crossreacting anti-TNFR IgG were detected by ELISA with TNFR (Sigma) or using synthetic P1 peptide versus P2 control peptide, with or without a common CagA/TNFR sequence, respectively (Lipopharm, Gdańsk). Pro-inflammatory potential of anti-P1 IgG-P1 complexes was evaluated in complement binding assay.

Results

In all *H.pylori* infected animals both anti-GE IgM and IgG antibodies were raised. The highest levels of anti-GE IgM were detected during acute whereas anti-GE IgG during chronic phase of infection, 7 or 28 and 60 days from inoculation, respectively. Anti-CagA IgG were induced in 11 *H.pylori* infected animals: 7 and 28 days post infection (4/10 and 7/20) but not 60 days from inoculation. Serum samples of anti-CagA IgG producers reacted in the ELISA with the complete TNFR (1/10, 7 days from inoculation; 5/20, 28 days from inoculation). All *H.pylori* infected animals, 7 and 28 days, but not 60 days from inoculation, responded by increased anti-P1 IgG production as compared to non-infected animals. Absorption of anti-P1 IgG positive sera with heat inactivated *H.pylori* resulted in elimination of *H.pylori*-driven anti-P1 IgG. Anti-P1 IgG-P1 immune complexes were able to activate complement indicating the pro-inflammatory potential of such complexes.

Discussion and conclusions

During *H.pylori* infection CagA protein may induce antibodies crossreacting with host TNFR. This may result in the maintenance of inflammatory process due to complement dependent cytotoxicity or modulation of TNFR-dependent cellular responses.

The utilization of the *in silico* methods for the description of structure, functions and protein-protein interactions on the example of Kirsten rat sarcoma viral oncogene homolog (K-Ras)

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Background

The most popular databases offer abundant datasets describing protein structure and function, as well as proved or predicted protein-protein interactions. In most cases profound *in silico* analysis underpin the further design of the research. With that in mind, data presented here has been obtained employing selected bioinformatics software available online, commonly used for the description of structure, functions and protein-protein interactions, working on the example of Kirsten rat sarcoma viral oncogene homolog (K-Ras).

Material and Methods

Information about the protein of interest was obtained from the online open-access databases: UniProt, SCOP, Genebank, GeneCands, BioGrid, IntAct, STRING and OMIM. 3D Simulation of the quarternary structure has been generated with RSCB PBD software. Data were supported with a literature review conducted with the use of databases such as Science Direct and PubMed.

Results

KRAS is usually tethered to cell membranes because of the presence of an isoprene group on its C-terminal hypervariable region (HVR), whereas the catalytic G domain is localized at the cytoplasmatic site [1]. The K-RAS protein is GDP/GTP-binding protein that acts as an intracellular signal transducer, being activated by a guanine nucleotide-exchange factor (GEF) and inactivated by a GTPase-activating protein (GAP) [2]. K-Ras is involved in numerous cellular pathways, including proliferation, differentiation, and senescence [3].

The GTPase activity of K-RAS is significantly enhanced by recruitment RasGAP. Henceforward, K-RAS can bind to SOS1 (representative of GEFs class), which forces the release of bound nucleotide (GDP) [4]. Subsequently, K-RAS binds GTP present in the cytosol and the GEF is released from ras-GTP. After allosteric activation, K-RAS recruits and regulates proteins essential for the propagation of growth factors, as well as other cell signalings receptors like c-Raf and PI 3-kinase. K-RAS is also involved in MAPK pathways. Moreover, K-RAS influences gene expression i.a. through positive regulation of NF-kappaβ transcription factor activity [5-10]. While wild-type K-Ras protein plays a vital role in normal tissue signaling, mutated genes are potent oncogenes. Mutation at the active site has been identified in about 20% of human cancers such as lung adenocarcinoma, mucinous adenoma, ductal carcinoma of the pancreas and colorectal cancer [11].

Discussion and conclusions

K-Ras has been proven to be involved in numerous crucial cellular pathways. Mutated protein isoforms behave differently presumably due to differences in the C-terminal hyper-variable regions. While highly recurrent in cancer, attempts to target these RAS mutants with inhibitors have not been successful, and has not yet become common practice in the clinic. With that in mind, non-direct approaches targeting crucial protein-protein interactions of K-Ras are worth attention in further research, highlighting the role of thorough *in silico* analysis.

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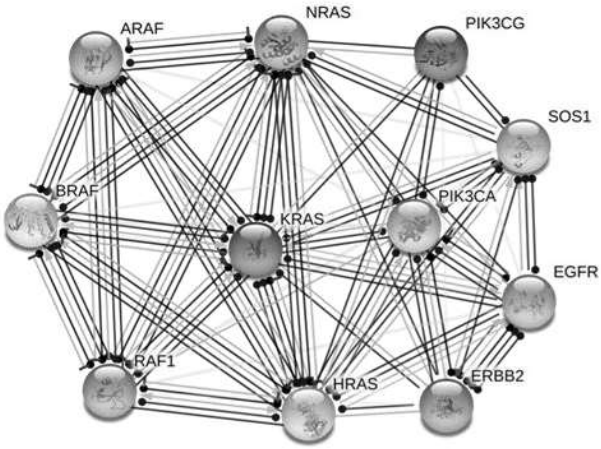

Fig 1 Protein-protein interactions network generated with STRING software;
►positive, ▌negative,● unspecified.

Liposomal formulation of curcumin for human pancreatic cancer therapy

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Background

Pancreatic cancer is one of the leading cause of cancer death worldwide. The survival rate is poor and depends on general patient's health. Currently the only realistic and possible treatment for fully recover is surgical resection followed by chemotherapy. Due to unclear early symptoms most of patients are diagnosed with an advanced stage or metastasis when resection option is impossible. The pancreatic microenvironment is very heterogenic. It is characterized by high amount of extracellular matrix components such as hyaluronic acid,

collagen or fibronectin. Because of that the diffusion of the drug into tumor is limited. Over the last few years attention of researchers is focused on natural substances with anticancer properties delivered by nano systems. One of biologically active compound is curcumin which demonstrate anti-inflammatory, aniti-oxidant, anti-proliferate and pro-apoptotic properties. Curcumin can modulate multiple signal cascades and pathways such as p53 pathway, STAT pathway or EGFR signaling pathway. Moreover curcumin acts as inhibitor of nuclear transcription factor (NF-κB) what can lead to increase of chemo-sensitivity of cancer cells. The anti-cancer potential of curcumin cannot be fully utilised due to its physico-chemical properties such as hydrophobic character and low bioavailability. The aim of this study was to increase solubility and uptake of curcumin. The cytotoxic effect of obtained liposomes with encapsulated curcumin were tested on pancreatic cell lines (BxPC-3, AsPC-1) and normal cell lines (NHDF).

Material and Methods

Curcumin liposomes were prepared by passive loading method using extrusion technique. Curcumin and lipids (DPPC/ SM/DSPE PEG-2000) were dissolved in chloroform. Curcumin was mixed with 20 mg of lipids in glass tube in weight ratio 1:10 respectively. Large MLVs were extruded through 200 nm Nucleopore polycarbonate filters in water bath at 64^{o}C. Then liposomes were centrifuged at 13000 RPM for 3 minutes. The size and polydispersity were determined using a Zetasizer Nano ZS. Concentration of curcumin was measured photometrically at λ = 425 nm. The lipid concentration was determined using the Stewart assay protocol based on the ability of phospholipids to form a complex with ammonium ferrothiocyanate. The cytotoxic effect of liposomal formulations were assessed by the MTT assay. Investigated pancreatic cell lines AsPC-1, BxPC-3 and normal cells NHDF were seeded into 96-well culture plates. All the lines were treated with 0-40 uM of curcumin loaded liposomes and incubated for 72 hours. The absorbance of the samples was calorimetrically measured at 560 nm with the reference wavelength of 630 on a microplatereader.

Results

The lipid composition of tested liposomes were DPPC/SM/ DSPE PEG-2000 in molar ratio 6,3/3,2/0,5. The obtained liposomes were in range from 130 to around 140 nm with polydispersity index ranging from 0,039 to 0,049. The efficiency of incorporation of curcumin was around 50%. Tested liposomal formulation showed potential anti-cancer activity on both pancreatic cell lines: BxPC-3 and AsPC-1 and were less toxic to a normal cell line (NHDF). Liposomal drug delivery system improve bioavailability and circulation time of curcumin.

Discussion and conclusions

Our work demonstrated cytotoxic effect on tested pancreatic cell lines with lower toxic respond on normal cells. These results suggest that curcumin has higher tendency to accumulate in tumor cells than normal cell lines. It can be explained that curcumin binds to many proteins involved in pancreatic cancer. Moreover curcumin inhibit production of type I and III of collagen which occurs in high amount in investigated type of cancer [2]. Curcumin is well documented compound which shows multiple actions on mutagenesis or apoptosis. Liposomes as a drug delivery system may increase the bioavailability, stability and due to small size of particles accumulation of curcumin in tumor cells. This study suggest that curcumin loaded liposomes could be a promising approach of treatment for highly aggressive pancreatic cancer.

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If SiO_2 nanoparticles are hemocompatibility for red blood cells *in vitro*?

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Background
Mesoporous silica nanoparticles (MSNs) are propitious candidates for nanoscale drug delivery systems due to their unique characteristics including biodegradability, changeable pore size, mesoporosity and high drug loading capacity. SiO_2 nanoparticles gain considerable attention as competent, safer and effective drug delivery vehicles due to their mechanical, chemical and thermal characteristics. The goal of this studies is to present the hemocompatibility evaluation of silica nanoparticles using red blood cells, a procedure which is a widely welcomed test to ensure the safety and compatibility of MSNs with biological systems [1, 2].

Material and Methods
The hemocompatibility and cytotoxic effects of SiO_2 were determined after exposure to different concentrations (0-200 μg/ml) at 2h and 24h. The hemolytic and osmotic resistance assays were described by Cyboran et al. (2012) with a minor modification [3]. The hemolytic activity of the compounds was determined on the basis of the concentration of hemoglobin that was released from erythrocytes after treatment with silica nanoparticles. In the osmotic resistance assay, a red blood cell suspension containing SiO_2 nanoparticles at 20 μg/ml and 50 μg/ml concentration was used. The impact of nanoparticles on the shape of erythrocytes was determined using an optical microscope.

Results
Mesoporous silica nanoparticles are safe for red blood cells in appropriate concentrations. Up to the concentration of 50 μg/ml and 2h time of incubation, they show good hemocompatibility. On the other hand, after 24h incubation of erythrocytes with silica nanoparticles, the increase of hemolysis process and decrease of osmotic resistance of red blood cells was observed. The shape of erythrocytes was changed after treatment red blood cells with SiO_2 NPs from native biconcave disc (discocytes) to echinocytes.

Discussion and conclusions
Hemocompatibility is mainly an *in vitro* test performed to evaluate the chances of test samples to cause unfavorable effects on red blood cells (hemolysis). To achieve the desired results, sample nanomaterials need to come in direct contact with cells and tissues. That is why the safe use of nanoparticles towards cells and tissues is the main problem in the nanodrug delivery system. It is comonly known that red blood cells are the first component in blood coming into direct contact with nanomaterials (because of its size and administration route). In case if the

adverse effect of silica nanoparticles takes place among circulating erythrocytes, it would be not important whether nanomaterials capable of transfer and freeing up pharmaceutical ingredients are used [1]. The results showed that SiO_2 nanopartciles (particle size < 20 nm) are safe for erythrocytes in concentration up to 50 µg/ml and show good hemocompatibility which makes them promising materials for future.

Acknowledgements
This work was supported from funds of the statutory activities of the Department of Physics and Biophysics, Wroclaw University of Environmental and Life Sciences, grants no. B010/0041/19.

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Fullerenes as targeted delivery systems used in antitumor therapy

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Anticancer drugs are cytotoxic for cancerous cells as well as the healthy ones. Cytostatic therapy may entail toxic effects and be inconvenient for the patient. Therefore, to increase the effectiveness and overcome the adverse effects, targeted cancer therapy is used. One of the possible methods of selective antitumor treatment is placing the drug in a nanocarrier [1]. That procedure improves the pharmacokinetics of the drug, increases accumulation at the site of uncontrolled cancer and also reduces the side effects of the drug [2].

The aim of this work was to gather information about promising role of fullerenes in the cancer treatment.

Fullerenes are an example of organic nanoparticle, which can be used as a delivery system. The most abundant synthetic allotropic carbon form is the fullerene form C60. It is a spherical particle with free space inside, characterized by a large ratio of surface to volume. C60 reacts easily with molecules containing electrons and is also able to bind to other compounds through chemical, physical and electrical interactions [3]. According to studies, conjugations of fullerenes with the drugs such as methotrexate, cisplatin, doxorubicin and paclitaxel exhibit potential applications in cancer therapy. Besides the enhanced efficacy of the cytostatics, those carbon nanoparticles also demonstrate the antitumor properties themselves. Fullerenes work by producing reactive oxygen species (ROS) that cause cancer cell death along with destroying ROS sensitive linkers resulting in the release of the drug and its action at the target site.

Along with the development of science and chemotherapy, many drug carriers have been tested, but fullerenes are the most prominent contenders due to their properties and structure [4]. It is believed, that fullerene drug delivery systems could be used as innovatory approach for treatment of cancer.

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Is it important which form of vitamin B12 to choose during supplementation?

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Background

Vitamin deficiencies are a common problem all over the world, including cobalamin, especially among those particularly at risk of low serum levels (vegans, alcoholics, patients with Castle factor deficiency). Cobalamin is a vitamin that takes different forms depending on the ligand attached to the cobalt ion in the molecule. This ligand may be a cyano (- CN), hydroxyl (- OH), methyl (- CH3) group, as well as 5'-deoxy-adensine. Although MeCbl and AdCbl have two different metabolic fates, different functions, and both are necessary, there is a paradigm for treating vitamin B12 deficiency only with methylated form. In addition, the majority of scientific research are works using only methylcobalamin. The aim of the study was to review the literature on metabolism, persistence and absorption of various forms of vitamin B12.

Material and Methods

The study analyzed selected papers publicshed in Pubmed databases in 1997-2019. By typing "cobalamine", 12044 items were displayed, of which limited to the most useful 18 English-language sources.

Results

The literature to date reports that the best treatment for cobalamin deficiency, in addition to intramuscular administration, is supplementation with both active forms at the same time. However, other sources say that regardless of what form they took orally, each of them is reduced to free form in the metabolic process. In addition, MeCbl and AdCbl are less stable against oxidation and temperature, while CnCbl is resistant to high temperatures. At the same time, there are reports that MeCbl is more efficiently utilized in the body compared to CnCbl.

Discussion and conclusions

The absorption of vitamin B12 takes place equally after consumption. Regardless of whether the vitamin is taken as methylcobalamin or cyanocobalamin, each of the cobalamin forms is reduced to the free form, which is Cbl, and cannot reach its destination, i.e. mitochondrion or cytosol, unchanged. The exception to this rule may be cases of mutation of the gene encoding MMACHC, which prevents metabolism of MeCbl, CnCbl or AdCbl. The thermal stability of CnCbl is higher compared to other forms, which makes it more attractive in terms of fortified food products. On the other hand, the study by Mayer et al.

Showed that methylcobalamin improved the concentration and quality of sleep of non-smoking patients who were not on a vegetarian diet faster than more effectively than cyanocobalamin.

In conclusion, the form of cobalamin taken does not seem to matter among healthy people (without MMACHC gene mutation), because each of the forms undergoes a reduction reaction under the influence of the MMACHC enzyme to a free form for further use. Treatment of vitamin B12 deficiencies with solely methylated cobalamin is therefore a controversial issue and requires more thorough scientific analysis.

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The influence of Abacavir on bone metabolism – preliminary results

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Background

In the recent years the length and the quality of life of patients infected with Human Immunodeficiency Virus (HIV) has increased due to development of antiretroviral therapy (ART). However, simultaneously, other health issues occurred, one of which is the higher risk of lower bone mineral density (BMD) and osteoporosis. Initiating ART has been shown to be the risk factor of foregoing[1]. Hence the great demand for examining the influence of drugs used in ART on bone metabolism.

Material and Methods

Research was conducted on fourteen male Wistar rats. They were randomly subdivided into two groups, each consisting of seven rats. Once daily group A received Abacavir 60 mg/kg body weight and group C (control group) received normal saline. After eight weeks the blood was drawn to conduct laboratory tests.

Results

After 8 weeks of the study no differences in the serum level of N-terminal propeptide of type I procollagen (PINP), osteoclast-deri-

ved tartrate-resistant acid phosphatase form 5b (TRACP 5b), total calcium, inorganic phosphorus nor creatinine were observed between both study groups (PINP: 3.74 ± 1.29 ng/mL vs. 4.18 ± 1.45 ng/mL; TRACP 5b: 1.38 ± 0.13 U/L vs. 1.49 ± 0.37 U/L; total calcium: 9.89 ± 0.12 mg/dL vs. 9.89 ± 0.23 mg/dL; inorganic phosphorus: 7.50 ± 1.10 mg/dL vs. 7.21 ± 0.88 mg/dL; creatinine: 0.30 ± 0.11 mg/dL vs. 0.34 ± 0.08 mg/dL, respectively).

Discussion and conclusions

Administration of abacavir for 8 weeks may not have negative impact on bone turnover in rats.

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Pancreatic surgery – a complex interdisciplinary problem

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The pancreas is one of the most important organs in the human body. It secretes hormones that regulate the work of the whole organism. It is responsible for the digestion of proteins and fats. In addition, it controls blood glucose levels. In recent years, we have seen an increase in the number of patients with pancreatic problems. The most severe are cancers.

According to data presented by the United European Gastroenterology Association pancreatic cancer is the 12th most common cancer worldwide and the 7th leading cause of cancer-related deaths worldwide. Additionally The American Cancer Society reports that in 2020 in the United States about 57,600 people will be diagnosed with pancreatic cancer and about 47,050 people will die because of it. Pancreatic cancer accounts for about 3% of all cancers in the US and about 7% of all cancer deaths. [1]

Surgical removal of the pancreas plays a key role in the treatment process, since it is impossible to remove the tumor without surgical intervention. It is also important that the patient undergoes surgery at an early stage of the disease. There are two types of surgery that can be used for such cancers: potentially curative surgery or palliative surgery. The first one is used when the clinical tests suggest there is a possibility to resect the cancer. The second one is used in situations when the cancer is too widespread and the operation is to relieve symptoms or prevent major complications.

The removal can be complete or partial and then it only affects individual parts of the pancreas: head, body or tail. The difficulty of the procedure is related to the location of the pancreas and the anatomical proximity of important organs and structures, such as the portal vein, liver, aorta or duodenum. Pancreatectomy involves the removal of surrounding tissues. In case of pancreatic head cancer, the most commonly used is Whipple procedure, which involves removing the pancreas head along the surrounding duodenum, the distal part of the bile duct together with the gallbladder, and the pyloric part of the stomach [2]. When the tumor is located in the tail or body of the pancreas, the removal affects not only these parts of the pancreas, but also the spleen.

Therefore, the result of surgery can be dysfunction not only of the pancreas itself, but also of nearby organs and tissues. Typical consequences of complete or partial pancreatectomy include deficiencies in endocrine

or exocrine pancreatic function requiring replacement of insulin or digestive enzymes.

Surgery, patient preparation and postoperative care is not only a surgical challenge, but requires the cooperation of many specialists. Such a procedure is burdened with many major complication [4]. As an important endocrine and exocrine organ, the secretory pancreas is both an endocrine and gastroenterological problem.

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Assessment of interaction isopropyl isothiocyanate and doxorubicin in human breast cancer cells

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Background

Moringa Moringa Oleifera has many therapeutic properties. Available studies show its cholesterol-lowering, anti-bacterial, anti-fungal and anti-inflammatory effects. It is suggested that it is a good source of antioxidants thanks to which it is to prevent the development of cancer, diabetes and heart disease. Chemical analysis has shown that the extract of this plant contains many vitamins, minerals, amino acids and fatty acids that have a beneficial effect on human health. For this reason, it is used in the kitchen and cosmetology.

Material and Methods

In the study, we focused on its cytotoxic properties. To this end, we analyzed the cytotoxic effect of doxorubicin and isopropyl isothiocyanate (Sigma), which can be isolated from Moring Oleifera. The concentration of isopropyl isothiocyanate were from 0,001 to 0,1% but doxorubicin were from 0,2 to 20 µg/ml. As a model *in vitro*, we used MCF-7/WT cells (breast cancer). We used the MTT test to assessed cytotoxicity after 48 and 72 hours.

Results

The results obtained show the cytotoxic properties of isopropyl isothiocyanate against human breast cancer cells.

Discussion and conclusions

Even though, the preliminary results are promising, further studies are need.

Acknowledgments: This research was supported financially by the Subsidy Funds of Department of Molecular and Cellular Biology SUB.D260.20.009.

Analysis of multiple risk factors for negative and/or indeterminate results of anti-Tick-borne encephalitis virus antibodies in human serum

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Background

Tick-borne encephalitis virus (TBEV) infections has been the cause of the threatening outbreaks for many years. In most patients infection results in full recovery. However, in 20-75% of them neurological symptoms has been reported. The infection may lead to meningitis, encephalitis, myelitis, central nervous system injury and fatal outcome [1].

Apart from several physical and chemical manners to prevent tick bites, active vaccination of people highly exposed to infection is still the most important strategy of prevention. Since basic vaccination does not provide the full immunization, several booster doses are recommended (once every five years in subjects under 60 years old and once every 3 years in subjects over 60 years old) [2]. However, in some subjects, the lack or low response to TEBV antigens is observed.

The aim of the current study was to assess the prevalence of non-positive results for anti-TEBV antibodies and the risk factors for wining immunity.

Material and Methods

2315 vaccinated subjects from the high risk group for TEBV infections participated in this study. All subjects were informed about the aim of the study and gave written consent for the participation. The study protocol was approved by the Bioethics Committee of Wroclaw Medical University (Poland). Data about age, sex and vaccination were acquired from written questionnaire due to participants` memory. Clinical samples of whole blood (to obtain serum) were collected. Serum aliquots were stored at -20°C before analysis. Commercial ELISA test was used for anti-TEBV IgG serum level assessment and results were defined as negative for values of <120 VIEU/ml, indeterminate for values between 120 VIEU/ml and 165 VIEU/ml, and positive for values of 165 VIEU/ml or more.

Results

Data showed that 86.2% of subjects who underwent vaccination were positive for anti-TEBV antibodies within 5 years. As much as 13.8% of subjects underwent to basic or basic and remaining vaccination were not protected barely after vaccination.

The analysis of time dependent frequency of non-positive results of anti-TEBV antibodies concentration (<165 VIEU/ml) showed that time elapsed since the last dose of basic vaccination was associated with increased number of negative results for anti-TEBV antibodies (Chi2 for trend, p=0.012). Data showed that the serum titer of anti-TEBV antibodies decreased during the time since the vaccination. In 27.3% of subjects vaccinated ≥4 years before testing and as much as 14.3% of participants underwent vaccination less than 1 year before testing, anti-TEBV titer did not reach 165 VIEU/ml. As much as 7.5% of subjects under 60 vaccinated <1 year before testing and 9.0% of subjects vaccinated ≥4 years before

testing had negative or indeterminate titer (<165 VIEU/ml) of anti-TEBV antibodies. A logistic regression showed that longer time since the vaccination doses constantly increased the odds ratio (OR 1.206; 95%CI:1.100-1.324) for non-positive values of anti-TEBV antibodies, when a higher number of booster constantly decrease the odds ratio (OR 0,573; 95%CI: 0.498-0.660) for non-positive values of anti-TEBV antibodies.

Discussion and conclusions

Some previous studies suggest that some subjects who received vaccination did not response to vaccination and some congenital disorders, might be a main cause of vaccination failure [3]. Severe cases of TBE has also been observed in patients previously vaccinated with initially proper response to vaccination [4]. For this reason, non-responders or low responders are not protected after primary infection or vaccination and lower titers of neutralizing antibodies delay the clearance of the virus and may result in infection of neuronal cells [5]. This study demonstrates that vaccination schedule should be personalized. The extension of the interval of booster immunization is risky and all subjects should be surrounded by care consisting of more frequent monitoring of serum antibodies by individual schedule to adjust the frequency of subsequent doses of booster vaccination.

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Innovative treatment of ischemic stroke's complications based on steam-cells therapy and cytokine administration

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Brain ischemic stroke is a serious medical condition which may lead to severe systemic consequences. In the vast majority of affected patients, it is followed by complications that lower the quality of their life. According to statistical data from 2015, brain strokes are the primary cause of disability among the middle-age group [1].

People who overcome brain stroke, report considerable deterioration of cognitive and motoric brain functions. The range and severity of impairment is adequate to the areas and regions affected by ischemic stroke. Nowadays, the most popular treatment method is rehabilitation. The aim of the method is to restore the full capacity of the movements and brain functions, in some cases even comparable to condition before damage.

However, in most cases, the effectiveness of rehabilitation is not high enough to satisfy patients and their demands. The full recovery is rarely achieved by this traditional treatment.

The application of stem cells, as well as cytokines with pro-regenerative properties gives a perspective that in the not far future patients suffering from ischemic stroke's complications may count on more reliable methods of treatment [2]. Aside from the low efficiency, the stem-cells based therapy may also be less inconvenient and time-

consuming. This means shorter recovery times and lower costs for the patients [3]. Proposed methods could be applied in the upcoming decade as a standalone or complimentary treatment method for the traditional physiotherapy.

In this project, the authors gather all the currently available data about stem-cells based treatment of ischemic stroke's complications. Presented data would show the perspectives of alternative to rehabilitation methods of recovery for patients suffering from stroke.

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Does ambient air pollutant PM_{10} effects on fetal growth?

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Background

Theme of publication 'Effects of prenatal exposure to ambient air pollutant PM_{10} on ultrasound-measured fetal growth" pointed out the problem of dependency between higher air pollution and fetal growth. Most of the studies were conducted in areas when air pollution level is lower (Europe, USA) and results were inconsistent. The hypothesis that was investigated exposure that the high level of PM_{10} (140,8 $\mu g/m^3$ in the current study) during pregnancy increases the risk of abnormal fetal growth.

Material and Methods

The study group consisted of 8877 pregnant women inhabited in Lanzhou, China between 2010-2012. Before studies, women were interviewed using a standardized and structured questionnaire to collect main information. Implemented ultrasound measurements (18 583) of four fetal growth parameters like biparietal diameter (BPD), femur length (FL), head circumference (HC) and abdominal circumference (AC).

Measurement of PM_{10} in Lanzhou have been compered with level of PM_{10} in Wroclaw, to estimate possibility of occurrence abnormal fetal growth in Poland.

Results

The results of interviews show that more than half of the women were younger than 30 (63,5 %), non-smoker (81 %), non-drinking alcohol (99,8%). Most of them had a normal BMI (68, 9%) and didn't have maternal diabetes (98,8%). Pregnant women were used mostly gas or electricity as a cooking fuel, which reduce possibility of different source of PM_{10}.

When the level of PM_{10} was exceeding over 150 $\mu g/m^3$, there were increases in standardized FL (P= 0.0012) and HC (P=0,0078) compared with lower levels. Correlation between increase PM_{10} (every 10 $\mu g/m^3$) and standardized BPD (P=0.0016) were noticed. HC measurement proved the highest dependency between PM_{10} level and abnormal fetal growth.

In Wroclaw average value PM_{10} in 2019 was 26 $\mu g/m^3$.

Discussion and conclusions

Study suggested that high level of PM_{10} during pregnancy increased the risk of occurrence abnormal fetal growth (over and under growth). Depends of the method which was chosen there are difference between degree of deviation from the norm. However, a small amount of similar researches does not allowed to unequivocal determining the correctness of the results.

Menace of abnormal growth in Poland is not as high as in China because the level of PM_{10} is significantly lower.

Preoccupation of this subject is very important, because of continuous growth of contents PM_{10} in inhaled air.

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Do-it-yourself artificial pancreas systems (DIY APS): non-commercial software for diabetes control improvement

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Type 1 diabetes (T1D) represents about 10% of all types of diabetes. The onset of illness is usually between the age of 10-14. T1D mainly affects children and young people (<30 years of age), the long-term duration of the disease is conducive to the occurrence of complications. The goal of diabetes management is to avoid the development of acute and chronic diabetes complications by maintaining glucose levels within the recommended values. [1] It is currently an incurable disease and has a huge impact on the daily life of patients. Appropriate motivation makes it easier to treat and to accept the disease. T1D is a disease that requires great self-discipline from patients, but does not stop them from living a normal life.

A slowdown of diabetes complications development can be achieved only through better glycemic control. However, recent studies indicate that a small number of people with T1D achieve their therapeutic goals. [2] The development of medical technology brings better and better devices for continuous glucose monitoring (CGM) and continuous subcutaneous insulin infusion (CSII) systems. The idea of automation the measurement of glucose and insulin supply, and thus creating an artificial pancreas, is developing. [3] While the hybrid closed-loop system (Medtronic MiniMed 670G) has been launched first to market, the community of people with diabetes and their families united online under hashtag „#WeAreNotWaiting" was developing non-commercial do-it-yourself artificial pancreas system (DIY APS). [4] The system initially created by few people grew in strength. Nowadays, several open-source software are available to make a closed loop by yourself. The number of users is constantly increasing, however the technology is unregulated. [3,4] Currently, only observational

evidence exists, but their results are promising. In surveys „loopers" reported e.g. more time in target glucose range, better sleep, less frequent hypoglycemia, improved HbA1c or even more confidence and fewer mood swings. [5] More reliable data is required to determine safety and outcomes.

The described technology is an example of a positive patient initiative that can improve the quality of care but omits the tests and regulatory steps required by the medical industry. There are many ethical and legal doubts about the participation of healthcare professionals in DIY APS usage. At present the software shouldn't be prescribed, promoted, initiated or recommended, but due to the spread of the system and promising data on its effectiveness, knowledge about it should be expanded.

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Fluoride content in spirulina (*Arthtospira* spp.) supplements from conventional and organic cultivation

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Background

Spirulina (*Arthrospira plantensis*) is a microalga with hypolipidemic, hypoglycaemic, as well as anticarcinogenic and anti-inflammatory properties. It is a rich source of proteins, vitamins as well as micro- and macro-nutrients, which makes it an increasingly popular dietary supplement. However, there is a risk that spirulina supplements may act as a source of fluoride in human nutrition. Significant intake of fluorine and the exposure to its low concentrations have negative effects on the human organism. The aim of the study was to determine the content of fluoride (F) in spirulina supplements, originating from conventional and organic farming.

Material and Methods

The material used in the study was 34 spirulina dietary supplements in tablet and powder form originated from traditional and organic cultivation. A total of 34 spirulina samples from different countries of origin were obtained from specialist shops. F concentrations in individual samples were measured by the potentiometric method with a fluoride ionselective electrode (Orion 9409 BN, Thermo Scientific, USA). The statistical analysis was performed using Stat Soft Statistica 13.0 and Microsoft Excel 2010.

Results

The F content in the supplements included in the study ranged from 10.526 ±2.12 to

165.805 ±5.22 ppm. Fluoride content in supplements in tablet form was significantly higher (p= 0.0241). No statistically significant differences in the F content were observed depending on the method of cultivation (conventional vs. organic).

Discussion and conclusions

The study demonstrated that spirulina cultivation method did not have a significant effect on fluoride concentrations, and supplements originating from organic cultivation did not have statistically significantly lower fluoride levels. On the other hand, a significant difference was observed in the fluoride content of supplements sold in tablet vs. powder form.

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Knowledge about coronary artery disease among Polish students – survey study

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Background

Coronary artery disease (CAD) is one of the most common cause of death in Poland. Nevertheless, the level of knowledge about this pathology, risk factors and complication seems to be insufficient in our society. The aim of our study was to investigate the level of knowledge about CAD among Polish students.

Material and Methods

We conducted a survey study by internet questionnaire. Interviewees were 173 students of Polish universities. The questionnaire was prepared in accordance to second version of Coronary Artery Disease Education Questionnaire (CADE-Q II). There were 31 questions that assess students' literacy, in each four options to choose: right answer (for 2 points), half-right answer (1 point), wrong and „I don't know" answer- both marked as 0 points. The maximum overall score of the test was 62 points. Statistical analyses were performed with the Statistica 13.1 (StatSoft, Statistica 13.1, Tulsa, Oklahoma, USA) software. Continuous variables are expressed as a mean ± standard deviation or median (interquartile range) and categorical variables as a number (percentage). Continuous variables were first checked for normal distribution by the Shapiro-Wilk test and then were compared by Student's t-test or U-Mann Whitney test if distribution was normal or different than normal, respectively. Categorical variables were analyzed using the chi-squared test or Fisher's exact test. All independent variables associated (P <0.2) with the score of questionnaire in an univariate model and not correlated with another independent variable were then included in the multivariate linear regression analysis to determine the score of survey. Two-sided P-value of less than 0.05 was considered statistically significant.

Results

We collected answers from 173 participants. Among them, there were 60 men (34.7%). The mean age of contributors was 22.0 (21.0-22.0) and the mean overall result of the survey was 48.0 points (44.0-52.0). In the questionnaire, 118 participants declared the contact with cardiovascular diseases (CVD) that was defined as their own illness or their family members or friends being affected. Surprisingly, in direct comparison of both groups – the students who had contact with CVD and who not had, there were no significant differences in terms of gender, age, the place of residence and the sum of the survey. The trend to higher self-assessment of knowledge was observed in contributors who had contact with CVD (P=0.06). By multivariable analysis, the younger age (β=-0.87, P=0.001; β – standardized linear regression coefficient) and higher self-assessment of knowledge (β=2.58, P=<0.001) was independently associated with higher overall survey score.

Discussion and conclusions

The knowledge about CAD in polish students may be considered as insufficient, Unfortunately, the personal contact with CVD did not correlate with higher CAD literacy. Further CAD awareness campaigns are necessary to gain adequate knowledge about CAD in Polish students.

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Dietary habits of children and adults

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The recent studies show that dietary habits of both children and adults depart from the recommendations. In comparison with other European countries of similar socio-economic status, the number of deaths and incidence of chronic diseases due to poor diet in Poland is increasing. The studies published by the Developmental Period Medicine on food products popular among children and adolescents (between 1 and 18 years of age) list fries (27%), chocolate (27%) and pizza (23%) to be the most popular. The study also revealed a highly worrying negative correlation between the age of the children under study and the consumption of food products containing sugar, glucose and high-fructose corn syrup – all of which increase the risk of obesity and diabetes type 2. Given the age of the children under study, the main potentially harmful food additives are: salicylates, monosodium glutamate, sodium benzoate, potassium sorbate, iron sulphate, ammonium sulphate, BHT and calcium disodium EDTA. Another study conducted on adolescents between 13 and 15 years of age shows the popularity of highly processed food products among this age group. Fast-food

snacks and sweetened carbonated beverages are consumed several times a week (33%) and several times a month (60%). In turn, the consumption of sweetened carbonated beverages (1 litre per day) was declared by 70% of the adolescents under study.

The study on dietary habits of adults found that the frequency of consumption of milk, brown rice, whole grain noodles and rolled oats decreases with age. Conversely, the consumption of salt, coffee and offal showed an increase with age of the respondents. These findings were also confirmed by anthropometric measurements, as the body mass index and the percentage of body fat showed an increase with age. According to the data by WHO, 650 million of adults and 340 million of adolescents suffers from obesity worldwide. The statistics are worrying as even among the patients with morbid obesity awaiting bariatric surgery, the dietary habits showed numerous shortcomings. Among such patients, the frequency of consumption of animal fats (lard, fatback), energy drinks and beer was higher with an increase of waist-hip ratio (WHR).

Keywords: additives, highly processed food, nutrition

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KIDMED test – a tool for children's diet assessment

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Background

The mediterranean diet is indicated as an ideal dietary pattern characterized by large intake of vegetables, fruits, bread and other forms of cereal, rice, beans and nuts. It also includes virgin olive oil as the principal source of fat, moderate amounts of dairy products and fish, and red meat in low amounts. The mediterranean diet provides most of the recommended macro- and

micronutrients in the right proportion, as well as antioxidants, and has positive effect on health by reducing the risk of wide range of chronic diseases such as myocardial infarction, diabetes, cancer, arthritis, and other pathologies related to oxidative stress. Dietary habits are shaped in childhood and unfavourable diet may result in negative long-term health consequences, therefore it is of utmost importance to promote healthy lifestyle among children. The aim of this study was the assessment of adherence to mediterranean diet among primary school children.

Material and Methods

The observational study was conducted among school-age children from a primary school in Wałbrzych. The study group consisted of 75 children (45 boys and 30 girls), mean age was 8 years old ± 11 months. Under supervision of teachers and parents children completed a 7-day dietary diaries by colouring and describing composition of their diet for a whole week and for every meal.

Obtained data were analysed with the use of the KIDMED test with a presumption that mediterranean diet can be treated as a model of a healthy and well-balanced diet regardless geographical region.

The KIDMED test (Mediterranean Diet Quality Index for children and teenagers) is a tool to evaluate the adherence to the mediterranean diet for children and youths and consists of 16 yes or no questions (Table 1). For each "yes" response one point is given to answers representing positive food habits, and one point is subtracted for those representing negative food habits. Three categories of adherence were defined:

poor – score ≤ 3 points

medium – score 4-7 points

high – score ≥ 8 points.

Results

Mean scoring was 3,09 points, the median was 3 points. In 45 children (60%) the adherence to model diet was assessed as poor, in 29 (38,67%) as medium, and only in one child as high. There was no significant difference between boys and girls.

Table 1. KIDMED test questions and scoring

KIDMED test	Scoring	
a fruit/fruit juice every day	+	1
a second fruit every day	+	1
vegetables regularly once a day	+	1
vegetables more than once a day	+	1
fish at least 2-3 times per week	+	1
fast-food more than once a week	-	1
legumes more than once a week	+	1
pasta/rice 5 or more times per week	+	1
cereals or grains for breakfast	+	1
nuts at least 2-3 times per week	+	1
regular use of olive oil at home	+	1
skipping breakfast	-	1
a dairy product for breakfast	+	1
commercially baked goods or-pastries for breakfast		1
two yogurts and/or some cheese daily	+	1
sweets and candy several times every-day		1

Discussion and conclusions

Our results indicate, that diet of 60% participants was assigned as poor, which corresponds with several studies reporting both low adherence to mediterranean diet among children (from 21-27% to 73.9%) and insufficient knowledge about healthy diet and role of fruits and vegetables consumption. This unfavourable trend is common to numerous countries. Although the KIDMED test is designed for mediterranean region, its usage in epidemiological studies becomes more popular also in other European regions. It can be also implemented in

nutrition education programs aiming to establish healthy eating habits at young age that will have beneficial effects in later life, and in prevention strategies for reducing childhood overweight and obesity.

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The advanced wound care device comprised of a porous matrix of bovine tendon collagen and glycosaminoglycan

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Background

Burns are known to be the most devastating injuries found in medicine. Despite the huge development in the process of wound care, the results of treatment are still not satisfactory.

Material and Methods

The paper presents treatment options using a matrix to regenerate the dermis.

Results

The biocompatible matrix for dermis regeneration allows the patient's body to rebuild its own skin. It comes in two forms: as a single or double layer membrane system. The first layer is composed of bovine collagen, surrounded by glucosaminoglycan (GAG), the second layer iscomposed of silicone. The matrix, after implantation in the wound bed, is colonized by host cells. In the first stage, monocytes and neutrophils produce and secrete cytokines, stimulating chemotaxis of endothelial cells forming a network of new blood vessels. Then migrating fibroblasts, producing and secreting proteins and proteoglycans, initiate so-called "Remodeling" – matrix reconstruction and reconstruction of the primitive dermis structure. At the same time, the collagen matrix is gradually biodegradable. The structure of the first matrix layer is a scaffold for the migration of fibroblasts, macrophages, lymphocytes and endothelial cells of capillaries, forming a network of new vessels. In the healing process, the collagen and GAG layers are resorbed and the new collagen is synthesized by fibroblasts, which lasts about 3 weeks. After this period, if a well vascularized new dermis is formed, the protective silicone layer is removed, and in its place an autogenic thin epidermal transplant is performed, whose cells expand to form mature epidermis covering the entire wound surface. As a result, the defect is replaced with functional dermis and aesthetic epidermis.

Discussion and conclusions

The use of a matrix to regenerate the dermis is especially important in extensive burns. Despite the fact that the first surgery with the use of matrix in Poland was carried out some time ago, because of the costs the treatment is not available in all centers dealing with burnt patients.

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Fabrication of Drug loaded Micro-needles for effective muscular pain relief

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Background

Microneedles (MNs) have wide applications for drug delivery as they are minimally invasive to the skin, creating small size pathways that deliver drugs directly below the stratum corneum without having to penetrate the upper layers [1]. This can potentially be used to treat conditions requiring instant relief or sustained effect. The minimally invasive nature of MNs improves acceptability and patient compliance. This project proposes to use MNs comprising a suitable combination of muscle relaxant to give instant pain relief and NSAID to provide sustained release [3].

Material and Methods

We needed to fabricate microneedles with precision up to the micrometre. For this we used two methods of making high precision molds. The first method involved using a micro-CNC machine to make positive molds of stainless steel. The second method involved using the Nanoscribe 3D printing machine, University of Alberta [2]. The drugs selected were diclofenac sodium, and thiocolchicoside. The hyaluronic acid (HA) dissolving MN patches were fabricated by a two-step micromolding process. The MN master molds were used to prepare PDMS female molds as per the manufacturers. HA aqueous solution was poured over the female molds, followed by vacuum treatment. The samples were dried in a sealed desiccator overnight at room temperature. After being peeled off from the female molds, the final MN patches were sealed and stored in the desiccators at room temperature and protected from light. The two drugs were then incorporated into the HA dissolving MN patches at various drug concentrations. They were subsequently detached from the molds and stored appropriately.

Results

The first method using Nanoscribe gave us only the top needle layer as the machine had size limitations. It included preparing data for exposure, tool loading and unloading, specimen development and post develop-

ment UV curing. We also used an adhesive to attach it to the rest of the positive mould made on a regular CNC machine. In the second method we used a simple Micro-CNC however the accuracy of the mould was not upto the mark. We used PDMS to make the negative molds and poured the dual drug loaded polymer solution in this mould to develop the MNs. The MNs were evaluated for mechanical properties and drug release profile using Franz diffusion cell. The mechanical strength showed an inverse relationship with drug loading. The release studies indicated an initial release of around 50-60% attributed to the water-soluble nature of HA. About 75% and 85% of drug in the MNs was released in the medium at 30 min and 60 min respectively.

Discussion and conclusions

We tried two ways of making the positive molds of the MNs, the accuracy was important as every patch made should have the same amount of drug. Using the Nanoscribe machine included an extra step of using an adhesive giving an accurate needle mold. Using the micro-CNC machine gave us a singular block but with lower accuracy. The mechanical strength of MNs is affected by tip radius, material composition and geometry as observed in this study. The dual drug loaded MN delivery system could serve as a promising technology alternative for effective delivery of dual drugs in the treatment of muscle pain circumventing the gastrointestinal side effects of NSAIDs.

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Calcium electroperation for prostate cancer tratment – *in vitro* studies

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Background

Calcium electroporation (CaEP) proved its effectiveness in numerous *in vivo* and *in vitro* studies. Currently the first clinical trials are onging. The aim of this research is to explore the possibility of calcium EP on prostate cancer.

Material and Methods

The Du-145 prostate cancer cells ware elctroporated in HEPES buffer with 8, 100 µs pulses of 400 to 2000 V/cm and incubated for short time in different calcium concentrations: 0mM, 0,5mM, 1mM, 2mM, 5mM and 10 mM. Secondly, with optimized parameters, the cell electroporation with calcium was performed. The cell viability was measured with MTT assay. Cell permeability assay with Yo-Pro-1 dye was performed with flow cytometry. The cell motility after CaEP was investigated with scratch assay.

Results

The optimal reversible electroporation was achieved when 800 V/cm pulses ware applied. The CaEP has significantly decreased the prostate cancer cell viability.

Discussion and conclusions

The research shows that CaEP can be potentially used as an alternative for minimal-invasive focal therapy of prostate cancer. Moreover, we suggest that calcium ions can potentially strengthen the effect of already incorporated into clinical practice irreversible electroporation.

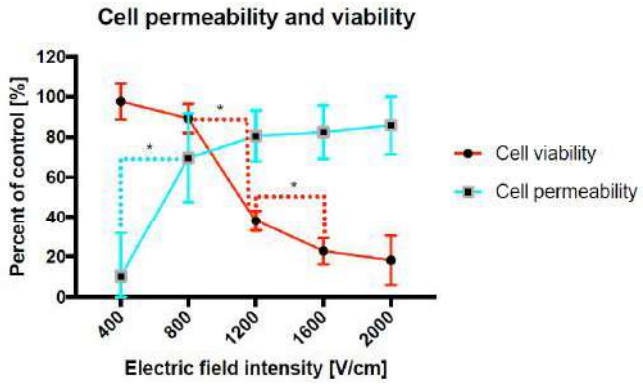

Fig. 1 Cell viability and permeability after electroporation without drug

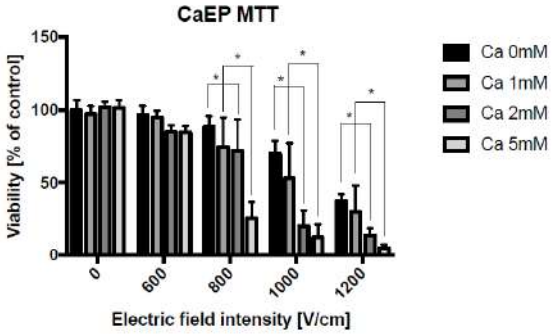

Fig. 2. Cell viability after electoporation with calcium in different concentrations

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Safety of using calcium supplements in people with casein allergy

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Background

Calcium is an essential component of the human body. It is mainly absorbed in the small intestine and is deposited in the bones by circulation [1]. One of the most important roles of calcium in the body is skeletal mineralization. Adequate intake of this element is important at every stage of human life. Normal peak bone mass achieved in adulthood minimizes the risk of osteomalacia and osteoporosis later in life [2]. Groups at risk of calcium deficiency include children and adolescents, the elderly, but also people who are allergic to cow's milk proteins [3]. The main milk proteins include casein. Casein increases calcium absorption but at the same time has a strong allergenic effect, which in the group of people with cow's milk allergy may even lead to anaphylactic shock [4]. Patients must completely eliminate dairy, which is the best source of calcium in the diet [3]. The elimination diet makes it necessary to take supplementation [5]. The source of calcium in dietary preparations is often not specified on the packaging, which makes it reasonable to suspect that the calcium in these products comes from dairy products. In addition, casein is resistant to high temperatures and it is difficult to remove it from all dairy products, and it may even be found in milk replacers with a high degree of hydrolysis.

The aim of the study was to evaluate that calcium supplements available in the pharmacy are safe for people allergic to cow's milk proteins.

Material and Methods

The study was carried out on a randomly selected sample of 21 calcium supplements by the enzyme-linked immunoassay method, using a casein test (kit test, produced by Neogen, Noack Polen District). Tested supplements were available without a prescription. Each sample was tested in two repetitions.

Results

All tested samples showed a concentration below the method's limit of quantification <2.5 ppm. These values also do not exceed the permissible amount of casein that can cause allergic reactions.

Discussion and conclusions

The report of the Supreme Audit Office showed that supplements may be contaminated and adulterated. Manufacturers often do not declare all substances used to prepare the supplement. Hence, patients may have various types of ailments, e.g. gastrointestinal, which will be difficult to identify. The possibility of adulteration or contamination of the supplement indicates the need to conduct the above tests regularly in order to maintain the highest safety of such preparations in patients [6].

The calcium supplements tested appear to be safe for use by people with a casein allergy. Each time the amount of casein in the samples was below the detection threshold.

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Nanomedicine for targeted treatment of tumor diseases

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Background

Cancer is the second leading cause of death in developed countries. It is known that standard antitumor therapy has a number of serious adverse biological effects. One of these is a lack of selectivity for tumor tissue, resulting in significant side effects. The relatively low therapeutic concentration of the active compound often results in drug resistance and multi-resistance of tumor cells.

Material and Methods

This review analysis (2000-2020) is focused on nanotechnology. The methodology of the choice used scientific studies from more than 500 viewed articles from databases MEDLINE, PubMed and Google Scholar based on the search phrases, such as nanomedicine, thiols, nanoparticles.

Results and Discussion

Nanotransporters for targeted treatment are a modern and effective way of personalized approach [1]. Carbon, gold, silver and other nanoparticles (NPs) can be used as the basis of the nanotransporter [2]. NPs can enter a cell independently of its type and functional group attached to the surface of the nanoparticle. Various *in vitro* and *in vivo* studies have shown that many functionalized nanoparticles are biocompatible [3]. The physico-chemical properties of nanoparticles play a decisive role in their potential toxicity [4]. For NPs, shorter and thicker nanotubes have been found to exhibit lower toxicity. Chemically functionalized NPs are much better water-soluble and have greater stability in the physiological environment. Attempts to use NPs to target multivalent ligands in cancer are increasing rapidly [5]. In addition to passive targeting methods based on the enhanced permeability and retention (EPR) effect and the specific acidic environment in the tumor, strategies for actively targeting a selected tumor using ligands or antibodies that increase the specificity of the nanotransporter are also investigated. However, a protein corona plays a major role in the application of NPs *in vivo* [6, 7]. A protein corona is a cluster of all proteins that can bind to NPs. Protein corona formation is usually associated with a significant reduction in therapeutic potential. Albumin is the most abundant component of the protein corona. It has been shown that the composition of the protein corona depends on the structure and physicochemical properties of the NPs. However, the effect of surfactants on the structure of NPs, on the composition and formation of the protein corona, has not yet been investigated. In our experiments, the effect of the interaction of serum albumin and NPs was studied. A completely unanswered question is the interaction of nanoparticles with thiol compounds such as low-molecular-weight glutathione or metallothionein.

In addition to the above, Giulimondi et al. observed increased expression of albumin receptors in some malignant tumors (liver, gallbladder, but also breast cancer)[7]. Protein corona-modified nanoparticles may be useful for targeting albumin receptor-overexpressed tumor cells.

Conclusions

This research area of nanomedicine is completely open and will certainly bring many unexpected discoveries in the near future.

Acknowledgements

The work was carried out with the support of the H2020 CA COST Action (CA15114) and International Collaboration Project of The European Technology Platform for Nanomedicine.

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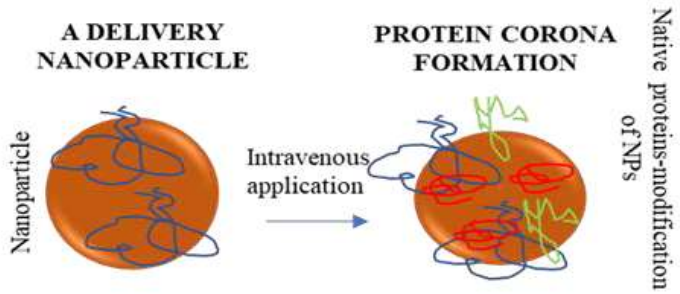

Fig. 1. The negative impact of the protein corona on the nanoparticle targeting ability. A target protein (blue), a native protein (red, green)

Cryoglobulins and their damaging effect on organs. Etiopathogenesis, symptoms and diagnosis of cryoglobulinemia

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Cryoglobulinemia is a rare disease (1 case per 100,000 individuals) consisting in antibodies which precipitate *in vitro* at low temperatures and disappear when incubated at 37˚C [1]. It is observed in the course of various disorders. Three types of cryoglobulinemia are identified on the basis of laboratory investigations: type I with

monoclonal immunoglobulins (IgG, IgM, IgA, or their κ or λ light chains, strongly connected with monoclonal gammopathies such as a monoclonal gammopathy of undetermined significance (MGUS) or a B-cell lineage malignancy), type II (most often) with a mixture of a monoclonal IgM with rheumatoid factor (RF) activity and polyclonal IgG (linked to hepatitis C virus infection) and type III with a mixture of polyclonal IgM with rheumatoid factor activity and polyclonal IgG (connected with autoimmune diseases like SLE) [2, 3].

Cryoglobulins cause tissue damage in the mechanism of: increasing blood viscosity (hyperviscosity syndrome), plugging and thrombosis of blood vessels (deposits in small arteries and capillaries) and deposition on the epithelium of blood vessels and the blood complement activation (the systemic vascular inflammatory reaction) [4]. Each type may be manifested by a different set of symptoms. Type I cryoglobulinemia is characterized mainly by the presence of skin lesions such as purple-colored papulae on lower limbs, livedo reticularis, Raynaud's phenomenon, ulcers and tissue necrosis. Types II and III more frequently present with symptoms within other systems, with strong muscle and joint pains becoming more intensive at lower temperatures, peripheral polyneuropathy, hepatic dysfunction, respiratory symptoms or membranoproliferative glomerulitis [1,5].

Diagnosis is based on clinical and laboratory findings. The detection of serum cryoglobulins is necessary for correct classification. It involves a blood test in which the sample must be kept at 37°C for a period of time before being cooled. After precipitation at 4°C and centrifugation measurement of cryocrit can be performed. Further steps (washing and prewarming of precipitate, electrophoresis, immunofixation at 37°C) help to type cryoglobulins [2, 3]. Treatment depends on underlying disease and includes e.g. immunosuppressors, corticosteroids and/or plasmapheresis.

The aim of this paper is to present etiopathogenesis, clinical features, diagnostic approach and treatment of cryoglobulinemia. Presented information may help clinicists diagnose this very rare disease.

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Synthesis of new pyrrolo[3,4-*c*]pyrrole derivatives with potential analgesic properties

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Background

Cyclooxygenases (COX) is a group of enzymes that play a large role in a biosynthesis of prostaglandins (PGE) and thromboxane (TXA_2). COX catalyses the conversion of arachidonic acid into those substances. Arachidonic acid derivatives have diverse physiological functions such as causing inflammation, blood clotting, pain or fever [1].

COX have two different isoforms. COX-1 is responsible for maintaining the proper function of internal organs, so inhibiting it may cause adverse effect such as kidney damage, gastrointestinal bleedings and ulcers. COX-2 stimulates PGE biosynthesis in inflammatory cells. Therefore it is crucial to develop Non-steroid Anti-Inflammatory Drugs (NSAIDs) selective towards COX-2 izoenzyme [2].

N-Mannich bases are promising group of chemical compounds with potential analgesic properties and high pharmacological activity [3]. In this paper we are presenting synthesis of new pyrrolo[3,4-*c*]pyrrole derivatives which may present those properties.

Material and Methods

The key substrates for the synthesis of the final compounds derived from the pyrrolo[3,4-c]pyrols were imides (butyl or phenyl substituent). These imides were obtained by process of multistage synthesis, the first stage of which was condensation that leads to obtaining diester. Next, condensation of diester with aniline/n-butylamine was performed. Intermediate product was then transformed into diacid during hydrolysis. Anhydride diaccid was converted into amide-acids. Finally, amide-acids underwent intramolecular cyclization, with the formation of imide. The imide constituted key substrate for the subsequent stage of synthesis [3].

In a reaction of the proper imide with formaldehyde, arylopiperidine derivatives (in ethanol) final compounds (Fig.1) were obtained.

Results

Initially, *In silico* computations were conducted to calculate estimated bioactivity. Molecular docking procedure was performed based on Lamarckian Genetic Algorithm (LGA) using AutoDock 4.2 program [4].

Derivatives with butyl or phenyl group (R^1) attached to nitrogen atom in pyrrole group, and with 4-chloro or 4-bromo (R^2) phenyl group attached to piperidine were obtained. General formula of these new compounds is presented on Fig 1.

The compounds were obtained with a very good efficiency (70-80%). Structures of those compounds were confirmed by FTIR and ^{1}H NMR.

Discussion and conclusions

Derivatives obtained during this synthesis may be promising path in further research on selective COX-2 inhibitors, but further tests are required to determine their pharmacological properties. Therefore, due to prospective results of Autodock computations and good efficiency of synthesis, we decided to send these compounds to *In vitro* cyclooxygenase (COX-1 and COX-2) inhibition assay and evaluation of viability.

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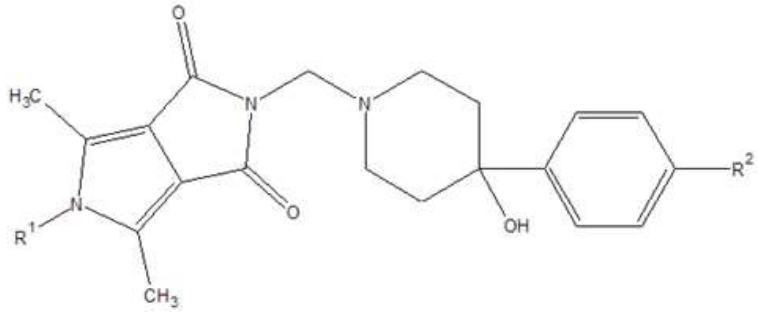

Figure 1. General formula of obtained compounds

R^1 = *-C_4H_9, -C_6H_5*

R^2 = *-Cl, -Br*

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Expression of selected inflammatory parameters as markers of endometriosis progression – pilot study

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Background

Endometriosis is a gynaecological disease, that pathogenesis seems to be strict associated with inflammatory processes. To evaluate the subclinical inflammation in the blood serum of women with endometriosis, we were tested C-reactive protein (CRP), immunoglobulin G (IgG), interleukin 1β (IL-1β), interleukin 6 (IL-6) and Human Chitinase-3-like Protein 1 (YKL-40), to find a non-invasive marker of endometriosis progression.

Material and Methods

The study group of patients consisted of 43 women with histologically confirmed endometriosis. The serum samples – material for the study - were collected at the Department of Gynaecological Oncology in Lower Silesian Cancer Centre (Poland). Based on the revised American Fertility Society (rAFS) classification, 20 women displayed moderate and 23 severe endometriosis (stage III and IV, respectively). We also included 19 women with no history of endometriosis as a healthy control group. In all samples we measured concentrations of: CRP, IgG, IL-1β, IL-6 and YKL-40. Statistical analysis was performed using Statistica PL version 13.3 (StatSoft Inc., Tulsa, OK, USA). The p value < 0.05 was considered significant.

Results

We observed a significant higher concentration of CRP in patients with IV stage of endometriosis in comparison to III stage of endometriosis ($p=0.021$). No significant differences were found in the concentration of serum IgG, IL-1β, IL-6 and YKL-40

between III and IV stage of endometriosis, however, we observed lower median concentrations of IgG and YKL-40 and higher median concentration of IL-1β and IL-6 in patients with severe endometriosis as against moderate stage. We observed significant difference in concentrations of CRP, IgG, IL-1β and IL-6 between group of severe endometriosis and the control group ($p=0.005$, $p=0.016$, $p=0.014$, $p<0.001$, respectively), whereas we did not observe any differences between III stage of endometriosis and control group. There was a positive correlation between CRP and IL-1β ($r=0.34$, $p<0.001$), CRP and IL-6 ($r=0.58$, $p<0.001$) and a negative correlation between IgG and IL-6 ($r=-0.42$, $p<0.001$).

Discussion and conclusions

The symptoms of endometriosis are often non-specific and may suggest many various gynaecological diseases. One of the reason of diagnostic delay of endometriosis confirmation is trivialization the symptoms of disease, not only by patients but also by gynecologists. Unfortunately, the laboratory medicine has not yet a noninvasive biomarkers that may diagnose and/or confirm the stage of endometriosis progression. It is clearly seen that diagnosis of endometriosis requires special algorithm with adequate medical procedure with specific biomarkers. One of the panels of such inflammatory biomarkers could be serum CRP, IgG, IL-6 and IL-1β which can support the diagnostics of endometriosis what is especially important in the stage of the disease.

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The importance of monitoring blood homocysteine levels in clinical practice

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Background

Homocysteine (Hcy) is an amino acid that includes thiol groups in its particles. It is formed in all cells of the organism as a result of the metabolism of one of the exogenic amino acids – methionine. The necessary cofactors for the biochemical course of changes of Hcy include: folates and vitamin B12 (the methylation process of Hcy into methionine), vitamin B6 (the catabolism of Hcy into cysteine), vitamin B2 (the process of forming 5-methyltetrahydrofolate by

means of 5, 10- methylenetetrahydrofolate reductase), betaine (the remethylation process), magnesium and lithium (adenosine triphosphate is present in the form of a complex with magnesium ions and lithium ions; this compound, which is high in energy, participates in the transformation of methionine into S-adenosylmethionine via methionine adenosyltransferase). The aim of the study was to evaluate the importance of monitoring blood homocysteine levels in clinical practice.

Material and Methods

The literature present in the PubMed and Embase databases has been reviewed.

Results

Homocysteine undergoes autooxidation, which leads to the formation of biologically active substances that participate in signaling pathways associated with increased cell toxicity, facilitating apoptosis, necrosis, the formation of blood clots and the amplification of oxidative stress. The autooxidation of Hcy thiol groups results in the formation of reactive oxygen species (ROS), i.e. hydrogen peroxide. Additionally, by reducing the activity of glutathione peroxidase and the redox potential, Hcy amplifies the effects of ROS. In consequence, lipids, proteins, carbohydrates and nucleic acids undergo oxidation, leading to endothelium dysfunctions, the damaging of blood vessel walls, the activation of platelets and the formation of blood clots. Hyperhomocysteinemia is also toxic for neurons and glial cells. Among other reasons, the toxicity is a result of the intracellular mobilization of Ca^{2+} and oxidative stress within the endoplasmic reticulum, leading to apoptosis, the rebuilding of extracellular matrix in the brain and endothelium dysfunctions. Furthermore, free oxygen radicals have the ability to induce the activity of the NR1 subunit of the NMDA receptor (N-methyl-D-aspartate), which leads to its increased sensitivity to stimulating amino acids (glycine, serine, glutamate), resulting in disorders in the integrity of the blood-brain barrier.

Discussion and conclusions

In physiological conditions, when vitamin B is properly supplied in the diet, the concentration of Hcy in the blood should be correct. An increase in the concentration of Hcy can often be the result of deficiencies of vitamins B6, B12 and folate. Apart from an improperly balanced diet, the increase of the level of Hcy in blood can be the result of diseases of such organs as kidneys or the thyroid, as well as other factors, such as neoplasms, psoriasis, diabetes, the use of some drugs (e.g. metformin), alcohol consumption, smoking, elderly age, menopause, achlorhydria with a low level of Castle's external factor, intestinal inflammatory diseases and surgeries of the digestive tract (e.g. bariatric surgeries). The factors that contribute to the increase of the level of homocysteine in blood also include gene polymorphisms, mainly in reference to methylenetetrahydrofolate reductase (MTHFR) – the key enzyme of the folate cycle. An increased level of homocysteine in blood correlates with the existence of specific pathological units, such as: cardiovascular diseases, atherosclerosis, a stroke, depression, Alzheimer's disease and osteoporosis.

In conclusion it is worth to consider a routine examine of homocysteine level, especially in people with diseases that co-occur with elevated homocysteine levels in the blood, such as atherosclerosis and depression.

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Intake of protein, antioxidant vitamins and cobalamin in patients after brain tumor surgery

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Background
Surgery is a strong stress factor that comes with inflammation. Metabolic response after surgery is associated with use of own energy source like proteins needed to wound healing. After operation it is observed higher levels of glucose, protein breakdown, loss of nitrogen in the urine and negative nitrogen balance [1]. The operational gesture is associated with increased protein catabolism. Depending on the severity of the procedure, the catabolic phase may last from 5 to 10 days [2]. That is why the proper protein nutrition of patients is so important. The aim of the study was to determine the intake of protein, antioxidant vitamins and cobalamin intake in patients after brain surgery.

Material and Methods
The study group included 30 patients of the Pomeranian Medical University – Neurosurgery Clinic in Szczecin admitted for brain tumor surgery. The study involved 15 women (average age 48.30 ± 14.53 years) and 15 men (average age 56.33 ± 14.67 years). Each patient underwent a nutritional questionnaire which was introduced into the 5D Diet nutrition program to determine the level of protein and individual vitamin intake. The results obtained were compared to the Nutrition Standards for the Polish Population.

Results
The average protein intake in both groups was higher than recommended by the standards. The intake of vitamin B12 and A in men exceeded the norm twice. The consumption of vitamins A, C, E in both groups exceeded accepted norms.

Discussion and conclusions
Higher protein intake in both sexes has a positive effect on the body's protection in the phase of increased postoperative catabolism and protein loss. In addition, higher intake of vitamins with antioxidant activity seems to have a protective effect on inflammation occurring during and after surgery.

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Light-activated antibacterial polymer coatings

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Background

Despite significant advances in medicine, nosocomial infections are still a serious problem. In Europe, 4.5 million patients are affected by hospital-acquired infection every year and nearly 40 thousand of them ends with the patient's death. This increases the duration of treatment and can cause hospitals overcrowding [1]. Possible way to protect against bacteria is using antimicrobial surfaces. One of the most promising solution is preparation of coatings able to produce singlet oxygen ($^1O_2$) as a germicidal agent. Singlet oxygen has been the subject of research for almost 60 years [2]. It is an excited form of molecular oxygen arising in photosensitization process which is successfully used in photodynamic therapy [3]. Because of its ability to bacteria killing, singlet oxygen is also used for coatings design. As a result of light exposition, particles of photosensitizer (e.g. dye) suspended in the polymer matrix generate singlet oxygen which reduces surface level of bacteria.

Material and Methods

Polydimethylsiloxane (PDMS, $(C_2H_6OSi)_n$) provided by Dow Chemical Company, thionine ($C_{12}H_9N_3S{\cdot}C_2H_4O_2$), tert-Butyl (TBA, $C_4H_{10}O$, 99%) and methanol (CH_3OH, 99,8%) purchased from Sigma-Aldrich were used to obtain composite coatings. PDMS:Thionine films were received by solution spin-coating method. Singlet oxygen photogeneration was observed using ultraviolet–visible spectroscopy (UV-Vis). Measurements were made in quartz cuvettes with laser at wavelength 532 nm and xenon lamp using respectively 1,3-diphenylisobenzofuran (DPBF) and α-Terpinene (both supplied from Sigma-Aldrich) as an oxygen indicators. Scanning electron microscope (SEM) was used to observe the morphology of prepared coatings.

Results

During UV-Vis measurements, observation of decreasing absorbance for singlet oxygen indicator was done at appropriate wavelength (410 nm for DPBF and 266 nm for α-Terpinene) which is caused by its oxidation. Both measurements lasted for 30 minutes with 5 minutes intervals. In case of DPBF oxidation process, absorbance decreased from 0,599 to 0,296. α-Terpinene absorbance decreased from 1,049 to 0,933. The morphology of the imaged surfaces was characterized by the presence of small, randomly distributed thionine agglomerates suspended in a PDMS matrix.

Discussion and conclusions

Obtained coatings showed good ability to singlet oxygen generation. Lower efficiency of photogeneration for test with xenon lamp is the result of its work in the full range of visible light (greater range of photosensitizer excitation). In the case of observation with laser, photosensitizer excitation occurs at a wavelength close to its maximum absorption, therefore the photogeneration efficiency is higher. The presence of thionine agglomerates in the polymer matrix may be the result of insufficiently long mixing time of the solution (thionine didn't

dissolve and disperse well). In order to confirm the effectiveness of the developed PDMS:Thionine films, appropriate microbiological tests are planned. Methods of preventing contagions based on use of antibacterial coatings may become in the future an effective tool in the fight against hospital infections.

This work was supported by European Social Fund in the framework of the project "Silesian University of Technology as a Center of Modern Education based on research and innovation" POWR.03.05.00-00-Z098/17.

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Inflammation induced by long-term acetylsalicylic acid supplementation affects density of vasoactive intestinal polypeptide-like immunoreactive (VIP-LI) nerve fibres in the porcine jejunum

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Background

Vasoactive intestinal polypeptide (VIP) is considered to be one of the most important substances involved in the intestinal regulatory processes. VIP is an inhibitory factor causing the smooth muscles relaxation, suppression of the gastric acid secretion and vasodilation of the submucosa [1].

Acetylsalicylic acid, also known as aspirin (ASA), is a commonly used drug with analgesic, antipyretic, anti-inflammatory and anticoagulant effects.

The aim of the present study was to determine the influence of high doses of acetylsalicylic acid on the enteric nerve fibres of the porcine jejunum.

Material and Methods

This study was performed on 8 immature female pigs of the Pietrain x Duroc breed (approximately 8 weeks old and 20 kg body weight). The first group consists of control gilts (n=4) received empty gelatin capsules orally and the second group – experimental (n=4) received gelatin capsules with acetylsalicylic acid orally 100 mg/kg body weight. After 4 weeks the pigs were euthanized. Following fixation and freezing section, double immunofluorescence staining was performed. Antibodies against the protein gene-product 9.5 (PGP 9.5) and against the VIP were used as primary antibodies. As secondary antibodies were used Alexa Fluor 488 and 546. Stained 14 μm sections were examined under Olympus BX51 fluorescence microscope. The evaluation of VIP-LI-positive nerve fibres within the wall of jejunum was carried out on the basis of the counting of all VIP-LI-positive nerve fibres per microscopic observation field (0.1 mm^2). Such an estimation was carried out in five sections per animal (in five fields per section).

Results
The present immunohistochemical studies revealed that in the jejunum of pigs treated with aspirin the distribution of VIP-LI nerve fibres were significantly altered. Inflammation induced by long-term administration of high doses of acetylsalicylic acid caused increase in the density of the VIP-LI intraganglionic nerve fibres.

Discussion and conclusions
Our study showed that after aspirin supplementation there was increased density of the intraganglionic nerve fibres immunoreactive to VIP-LI. Similar changes were obtained during previous studies [2, 3]. We can assume that enteric nerve fibres underwent adaptation to the induced pathological condition. Our findings of increased density of VIP-LI-immunoreactive intraganglionic nerve fibres as a consequence of aspirin-induced inflammation provide valid evidence of the important function of this peptide in neuronal responses to inflammation.

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This study was supported by the National Science Centre (grant no. 2018/29/N/NZ4/00348).

Optimization of the conditions of 3D bioprinting with the microextrusion method – the influence of the pressure on the viability of the cells

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Background
The 3D bioprinting with the use of live cells is the newest technique from the field of biomedical engineering. One of the most important points of the procedure is saving the cells and letting them stay fully functional in the obtained bioconstruct. The most popular method used in 3D bioprinting is microextrusion [1]. Nevertheless, independently of the used bioink dosing method, it has to be kept in mind that inside the cartridge act forces that interact straightly with the cells suspended into entire bioink[2]. While using microextrusion method with the change of pressure, we change the forces acting on the cells. In this way, we may control the conditions of cells after the bioprinting process [4]. In our work, we showed that each cell line that is used in bioprinting process should had individually selected pressure range [3].

Material and Methods
Cells in the number of $5 \cdot 10^5$/mL are suspended in 3% of alginate and are bioprinted with the use of BioX bioprinter and with the pressure in the range of 0-200kPa. After bioprinting cells in the carrier were diluted with the use of 5mL of 1xPBS. The

visualization of the viability of the cells was performed by the FDA/Pi staining (fluorescein diacetate and propidium iodide staining).

Results

The maximum pressure for human (HFF-1) and mouse (3T3-L1) fibroblasts as well as for mouse endothelial cells (BALB-5206) could not be determined for the nozzle 840 µm thus we expected to be above 200 kPa. When we change the diameter to 200µm the viability of the cells is above 80% when the pressure is below 190, 110 and 170kPa in the case of HFF-1, 3T3-L1 and BALB-5206, respectively.

Discussion and conclusions

We confirmed that cells viability is depended on used pressure and from the inner diameter of the nozzle used in the bioprinting process. We checked 3 cell lines and 5 inner diameters of the nozzle, and for each of the cell lines we designated the maximum pressure which may be used during bioprinting process and the cell viability won't fall below 80%.

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Source of financing

This study was financially supported by The National Centre for Research and Development STRATEGMED3/305813/ 2/NCBR/2017.

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Chimeric antigen receptor therapies (CAR-T) – current knowledge and perspectives

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The joint efforts of biotechnologists and clinicians in recent years have led to significant improvements in gene therapies [1]. Despite the initial difficulties associated with genotoxicity and immune responses, successful clinical trials have enabled the Food and Drug Administration (FDA) to the registration of the first treatment in 2014. The dynamic development of CAR-T is observed in hematological oncology, especially lymphoblastic leukemias. This method involves isolating T-cells from the patient's blood and modifying them *ex vivo* through a viral vector, mainly lentiviruses and gammaretroviruses. The main target is to transduce a gene of the antigen-binding domain, which is fused to an intracellular signaling domain that mediates activation and co-stimulation to enhance T cell function and persistence. Recombinant T-cells after infusion are able to recognize, among others, CD19 antigen on cancer cells, bypassing the MHC system, and eliminate them.

Investigators are intensely focused on better understanding and treating systemic toxicity of therapy because it is still a major problem. Optimizing treatment costs is also a stinging challenge because they are extremely high up to more than 0,25 mln $ per patient. Nowadays researchers are looking for new antigen targets that may allow curing also myeloid malignancies and

solid tumors. Additionally, there is a need for methodologies that facilitate CAR-T cells entry into large tumors and overcome tumor microenvironment signals that disarm T cells.

The aim of this project was not only to sum up the latest reports connected CAR-T and present its possibilities in clinical oncology but also to explain the molecular basis of this type of gene editing.

Acknowledgments:
The work was created as part of the activity of the Student Research Group "Biology of Cancer Cell" at the Wroclaw Medical University (SKN No. K 148).

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Synthesis, proapoptotic and MDR reversal effect of new terpenoid derivative in colon cancer cells

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Cancer cells often possess intrinsically or develop during treatment the set of features known as multidrug resistance (MDR). The MDR phenotype cells often manifest altered properties such as genome instability or loss of the cell cycle control points, which also hinders effective chemotherapy. Because of the frequent occurrence of the resistance caused by the activity of the MDR transporters, it is essential to find the effective and – at the same time cell-non-toxic – inhibitors of those proteins. Terpenoid derivatives, which contain a preserved β-cyclocitral system in their structure, exhibit a broad spectrum of biological activities.

Anticancer activity is usually connected with their ability to induce apoptosis. In our studies, the ability of terpenoid derivative TMPE (3-(2,6,6-Trimetylocycloheks-1-en-1-ylo) prop-2-enian ethyl) to induce apoptosis of LoVo, HT29 and LoVo/Dx, HT29/Dx was confirmed. It was also checked whether TMPE could change cytotoxic effect of doxorubicin in sensitive and resistant sublines. In the presence of TMPE cytotoxicity of doxorubicin was elevated, and its intracellular accumulation increased. In case of LoVo/Dx and HT29/Dx an obtained value of combination index (IC) indicated for a synergistic interaction between doxorubicin and TMPE.

Paraoxonase 1 activities in the serum of women with polycystic ovary syndrome

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Background

Paraoxonase 1 (PON1) is calcium-dependent enzyme involved in many functions in human body. Various hydrolytic activities of PON1 can be broadly grouped into three categories, namely arylesterase, phosphotriesterase and lactonase [1]. PON1 is synthesized by the liver and released into the serum, where is associated with HDL [2]. Changes in PON1 status are observed in different diseases among others in polycystic ovary syndrome. Polycystic ovary syndrome (PCOS) is an endocrine disorder, afflicting females of 18-44 age. This disease leads to infertility, insulin resistance, obesity, and cardiovascular diseases. Since 2003, criteria in accordance with the consensus of the European Society of Humane Reproduction & Embriology (ESHRE) and the American Society of Reproductive Medicine (ASRM) established in Rotterdam, according to which the diagnosis of PCOS requires the presence of 2 of the following 3 symptoms:

1. no or rare periods,
2. hyperandrogenism / hyperandrogenemia,
3. image of polycystic ovaries in ultrasound [3].

The aim of the study was to assay PON1 activities in the blood of women with PCOS.

Material and Methods

The study included 40 women with PCOS aged between 17 and 39 years old and 24 healthy women aged between 17 and 40 years old. The value of body mass index was similar in both groups and was ranged between 18.5 and 25 [kg/m^2].

The diagnosis of PCOS was formed on the Rotterdam criteria. In both groups smoking and alcohol abuse were among the exclusion criteria. The serum were collected according to the routine procedure after overnight fasting and stored frozen until assays. In the group of women with PCOS, the blood specimens were collected during the follicular phase (within 3 and 5 days of the menstrual cycle).

Phosphotriesterase activity was determined with paraoxon as a substrate, lactonase activity of PON1 was determined using dihydrocoumarin as a substrate and arylesterase activity of PON1 was determined using phenyl acetate as a substrate [4].

Results

In the serum of women with PCOS both PON1 activities: arylesterase (75.70±27.11 U/l) and phosphotriesterase (173.62±99.05 U/l) were statistically significant lower when compared to the group of healthy women (113.87±32.20 U/l and 205.21±80.21, respectively). However, PON1 lactonase activity was increased in the serum of women with PCOS (21.72±2.85 U/l) when compared to group of healthy women (18.17±2.95U/l). When we divided women with PCOS according to BMI value we did not found any statistically significant differences between women with PCOS with BMI<25 and ≥25. However in the group of healthy women with BMI≥25 lower PON arylesterase activity (99.38±27.68 U/l) was observed when compared to the group of healthy women with BMI<25 (124.21±31.19 U/l).

Discussion and conclusions

Changes in PON1 activities in the serum of women with PCOS can be associated with metabolic disorders.

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Is using the iQOS system safe for health?

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The purpose of our presentation is to evaluate the impact on the human body of alternative tobacco products. We pay special attention to the new product, which exist on Polish market for 3 years, it is the revolutionary iQOS. According to the survey on public opinion, it has the characteristics of a low-risk product.

The incineration temperature of tabacco is different than temperature of burning in traditional cigarettes or e-cigarettes. In IQOS system the temperature should not exceed 350ºC, for regular cigarettes temperature of burning tobacco is approximately 800ºC, and in e-cigarettes the incineration temperature is between 150ºC and 180ºC. According to many safety assessment studies of alternative tobacco products focuses particularly on additional substances such as allergenic fragrances or glycerol in electronic cigarettes or tar and carbon monoxide in traditional cigarettes.

Due to information, that the research was sponsored by the manufacturer, their credibility cannot be confirmed and compare with the safety of e-cigarettes, which are described in detail in the scientific literature.

In our presentation, we present the independent research of scientists from San Francisco, published in the British Medical Journal, which compared the effects of IQOS, e-cigarettes and traditional cigarettes on the body, by affecting the functions of endothelium of blood vessels and the impact on lung cells, showing their high toxicity. In addition, studies by other scientists show toxic inhibitory effects on macrophages comparable to traditional cigarettes. High levels of lactate dehydrogenase, interleukin 8 in airway epithelial cells and airway smooth muscle cells have also been detected, which may indicate pathological conditions.

Therefore, it seems interesting to examine public awareness of the risks associated with the use of the IQOS system. As our own work, we want to present the results of surveys conducted among potential users of the alternative IQOS system and learn about their knowledge about this product.

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Copper(II) coordination compound with tolfenamic acid

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Background

Our research is focused on obtaining new forms of drugs as potential more effective pharmaceuticals, exhibiting higher solubility, stability, bioavailability and low toxicity. Synthesis of salts and coordination compounds is one of the method of improving of weak solubility and biopharmaceutical properties of drugs.

Tolfenamic acid belongs to the fenamic acids group known as nonsteroidal anti-inflammatory drugs (NSAIDs). It acts as cyclooxygenase (COX) inhibitor and prevents formation of prostaglandins. Tolfenamic acid is known as a cure for migraine [1, 2].

Material and Methods

Studied copper(II) coordination compound was obtained in the reaction of copper(II) acetate with tolfenamic acid in methanol, in the form of green needle-like crystals. The crystal structure of the compound was determined using a four-circle single crystal X-ray diffractometer at the Faculty of Chemistry at the University of Wroclaw. The theoretical analysis of chemical bonds and intermolecular interactions was performed using QTAIM [3] and noncovalent interaction (NCI) methods [4].

Results

Copper(II) tolfenamate coordination compound crystallizes in *P*-1 space group in the form of dimeric molecular compound that is build up from two copper(II) centres coordinated by four tolfenamate ions and two molecules of methanol. Each of the anions is linked to both Cu(II) ions *via* carboxylate group acting as a bridge of copper centres. The coordination sphere of each Cu(II) ion can be approximated by the octahedron, where O atoms from tolfenamate ions are located in vertices of the square. The fifth coordination place of Cu(II) is occupied by a molecule of methanol, while the sixth one is directed toward the second Cu(II) ion from the dimer (Figure).

In the crystal structure the network of intermolecular weak interactions is observed.

Discussion and conclusions

In the literature there are known a few structures of meclofenamic acid coordination compounds with copper(II) and other transition metals [5].

The crystal structure studies and theoretical analysis of the copper(II) tolfenamate coordination compound can provide a significant insight into its properties and potential influence on organisms.

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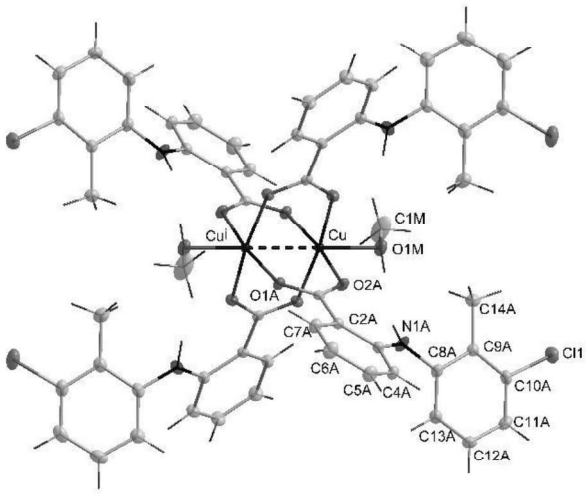

Figure. Crystal structure of $[Cu_2(C_{56}H_{44}Cl_4N_4O_8)(CH_3OH)_2]$

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Cardiomyocytes contractility improvement after treatment with 5-phenyloxyphenyl-5-aminoalkyl nitrate barbiturate and ML-7

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Background

Among the main factors contributing to the pathogenesis of heart during ischemia/ reperfusion (I/R) injury are an increased production of $ONOO^-$ and enhanced activation of MMP-2 [1]. It has been shown that oxidative stress during I/R induces phosphorylation and nitration/nitrosylation of myocardial contractile proteins such as MLC1 and MLC2 [2]. Phosphorylation and nitration/nitrosylation of MLCs increase their degradation by MMP-2, which leads to heart contractile dysfunction [3]. It has also been shown that $ONOO^-$ activates MMP-2 indirectly [4].

Previously we shown that administration of 5-phenyloxyphenyl-5-aminoalkyl nitrate barbiturate protects heart against I/R injury [5]. The aim of this study was to evaluate if administration of a mixture of subthreshold doses of myosin light chain kinase inhibitor (ML-7) and MMPs inhibitor 5-phenyloxyphenyl-5-aminoalkyl nitrate barbiturate has protective effect on contractility of I/R hearts. Also, to determine the effect of this mixture on levels of MMP-2 and MLC1.

Material and Methods

Cardioprotective effect of the subthreshold doses of drug cocktail was tested on isolated rat hearts by Langendorf method. Hearts extracted from anesthetized male Wistar rats (300-350 g) were perfused with Krebs-Henseleit buffer: after 25 min of aerobic

stabilization, hearts were subjected no-flow ischemia (20 min) in the presence or absence of inhibitors mixture (5-phenyloxyphenyl-5-aminoalkyl nitrate barbiturate (0.1 μM) and ML-7 (0.5 μM)) followed by 30 min of aerobic reperfusion. Next to hemodynamic parameters (coronary flow, heart rate, left ventricular developed pressure) biochemical markers of I/R injury were measured in a heart tissue and coronary effluents (MMP-2, LDH). The contractility of cardiomyocytes was measured using IonOptix Contractility System (IonOptix, Milton, MA, USA).

Results

Hemodynamic parameters of cardiac function were significantly reduced in hearts subjected to I/R compared to aerobic control. Administration of the drug cocktail improves all analyzed parameters ($p<0.05$). An increased activity of MMP-2 was demonstrated in hearts' homogenates as well as perfusates after ischemia/reperfusion in comparison to aerobic control. The usage of the mixture significantly decreased the activity of MMP-2 (in hearts tissue $p=0.0357$ and perfusates $p=0.0075$). Cardiac tissue damage induced by I/R was expressed by the release of LDH into the coronary effluent ($p<0.0001$). The results showed that administration of the mixture act caradioprotective and contributes to the improvement of cardiomyocyte contractility ($p=0.0004$).

Discussion and conclusions

We have shown, that co-administration of subthreshold doses of myosin light chain kinase inhibitor (ML-7) and MMPs inhibitor (5-phenyloxyphenyl-5-aminoalkyl nitrate barbiturate) improved cardiac mechanical function. Additionally, administration a low doses inhibitors mixture decrease production of LDH and prevents I/R induced increase in MMP-2 activity, protects MLC1 from degradation and improve cardiomyocyte contractility.

Study was supported by the National Science Centre grant no. UMO-2016/23/B/NZ3/03151

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Antitumor efficacy of ultra-short electrical pulses in murine colon cancer (MC38/0) *in vivo*

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Background

The application of ultra-short electrical pulses in cancer treatment is in research focus during past ten years. This technique is promising for tumor ablation without usage of cytostatic drugs [1]. Very short electrical pulses (3-600 ns) can be applied

with minimal Joule heating causing insignificant thermal effects in cells (temperature rise up to 3°C) [2]. Extracellular matrix, nerves, and vessels are preserved in the absence of heating, what promotes quick repopulation of the treated area with normal cells and fast recovery of treated tissues. Among other methods, nsPEF ablation is quite a new methodology and is subject for intensive study.

The aim of this study was the application of ultrashort pulses (10 ns) and high intensities of electric fields as the form of anti-tumor therapy in murine model of colon carcinoma.

Material and Methods

Murine colon carcinoma cells (MC38/0) were inoculated subcutaneously (s.c.), into right flank of C57BL/6 mice. When tumors reached a volume of 50 mm^3, nsPEF protocols were applied. We have applied 10 ns pulses with following parameters of nsPEF: 12.5kV/cm and 400 or 1200 pulses and 25kV/cm and 400 or 1200 pulses. After treatment the MC38/0 tumor growth inhibition was evaluated.

Results

The obtained results indicate the proportional decrease of tumor volume with the increasing electroporation parameters. The highest anticancer potency was obtained for 25kV/cm and 1200 pulses.

Discussion and conclusions

Ultra-short electrical pulses seems to be a promising and low invasive type of anticancer therapy. Similar studies were performed by Novickij et al. [3], where also 12 kV/cm electric field intensity was applied but longer 200ns pulses. Authors used myeloma tumor models and also observed decreasing tumour volume with the increasing parameters of PEF. Thus we can state that nanosecond irreversible electroporation can induce an anti-tumor response

Funding:

The research was supported by National Science Centre (Poland) within a framework of SONATA BIS 6 (2016/22/E/NZ5/00671; PI: J. Kulbacka)

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Effectiveness and toxicity of supplements containing red yeast rice and Monacolin K

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Background

Red yeast rice (RYR) is a food product used in the traditional Chinese cuisine. It is made from *Oryza Sativa* under the influence of *Monascus* yeast. The fermentation process enriches the rice with a compound that has a mechanism of action similar to that of statins – Monacolin K. Despite the identical structure the therapeutic effect of Monacolin K differs from the effect caused by

lovastatin. Differences in bioavailability and pharmacokinetics of those substances occur, what most probably results from the actions of other chemical compounds that are present in the–RYR. Increase in popularity of dietary supplements containing an extract from red yeast rice can be observed within the recent years. The substance is recommended as an adjuvant in the treatment of hypercholesterolemia. Red yeast rice is specially valued in the case of patients allergic to synthetic statins.

Material and Methods

Our poster shows various clinical trials focused on Monacolin K and its effects on health.

Results

The cholesterol-lowering effect of red yeast rice has been confirmed in several meta-analyses of randomized controlled clinical trials. One of the trials proved, that the intake of the substance within the time from 2 to 24 months reduced LDL-C by an average of 1.02 mmol/L compared to placebo, what is a similar effect as that of moderate-intensify statins.

Discussions and conclusions

However, some doubts concerning safe use of those substances occur. Monacolin is being sold as dietary supplements with less strict legal requirements. What is more, red yeast rice might be contaminated with citrinin – a nephrotoxic and hepatotoxic substance. Moreover its teratogenic effects has also been reported.

Monacolin K influences on the activity of liver enzymes what may lead to changes in metabolism of different drugs such as ciclosporin, fibrates, macrolides and verapamil. In the cases of particularly vulnerable patients monacolin K might cause myopathy and rhabdomyolysis. –Due to the two faces of Monocalin K, its supplementation must be controlled and and its toxic properties described in detail to avoid intoxication

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Steroid receptor RNA activator 1 in arteries

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Background

Steroid receptor RNA activator 1 (SRA1) is considered to be long noncoding RNA (lncRNA) [1] and functional RNA encoding the protein – Steroid Receptor RNA Activator Protein (SRAP) [2]. Both coding and non-coding SRA transcripts co-exist in human cells and acts as transcriptional regulator, multiple nuclear receptors, nuclear receptor co-regulators, and protein involved in gene silencing [3].

SRA1 is attributed to roles in regulating cellular response to estrogen and androgen stimulus, glucose uptake, the process of apoptosis, cell differentiation and proliferation, thereby affecting steroidogenesis, myogenesis, tumorigenesis and cardiomyopathy [3,4].

It is reported that SRA1 is highly expressed in liver, skeletal muscle, adrenal gland, and the pituitary gland; intermediate expression levels are observed in the placenta, lung,

kidney, and pancreas; in the brain and other typical steroid-responsive tissues such as the prostate, breast, uterus and ovary the expression is rather low [5]. This protein is also found in case of several human tumors types, mainly tumours of the breast, liver and bone [4]. The reports describe the high expression in smooth muscle, but they does not specify whether it is vascular smooth muscle or, for example, the digestive system muscles [6]. No information has been found in the literature on the subject whether SRA1 is present in the aorta and whether it has any significance in the pathomechanism of atherosclerosis. Our hypothesis is that it can control the migration of myocytes from the intima to the media and the proliferation of these cells, contributing to development of atherosclerotic plaque.

Material and Methods

The quality and semi-quantitative analysis of SRA1 content in 27 sections of the human aorta, which are at various degrees of atherosclerosis (according to the American Heart Association) was performed. This study was approved in terms of ethics by the Bioethics Committee of Wroclaw Medical University (no 577/2017). The samples came from people who died a sudden death (average: 68±15 years old). The standard immunohistochemical method was carried using anti SR-A1 rabbit monoclonal antibodies conjugated with HRP (1:100, Abcam, ab202922).

Results

The SRA protein is particularly visible in the area of the fatty core, in the region of cholesterol fissures formation. The antigen is visible in point form. In some patients, the antigen also occurs in myocytes of media altered by atherosclerosis. It can also be found in adventitious cells. Only trace amounts of antigen are observed in probes at low atherosclerosis level.

Discussion and conclusions

Up to date it is not possible to precisely define the function of SRA1. In the context of the impact of SRA1 on cell migration and proliferation, we have data showing that SRA can inhibit the proliferation of certain cells [4], but can also be attributed to the growth of others [7]. Reduced expression in some types of cancers [8] also suggests that this protein rather inhibits proliferation. We discover a tendency to increase protein expression in atherosclerotic plaque. This result suggests that SRA1 may contribute to the stimulation of migration and/ or proliferation of myocytes in the wall of large blood vessels such as the aorta.

As future direction we plan to examine content of noncoding and coding transcripts of SRA1 in the tissue.

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Voltammetric determination of fenamic acid on poly-*N*-acetylaniline modified glassy carbon electrode

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Background

Fenamic acid (FA) is a derivative of anthranilic acid. The compound is a parent structure for several non-steroidal anti-inflammatory drugs: mefenamic acid, tolfenamic acid, flufenamic acid and meclofenamic acid [1]. Due to its side effects fenamic acid is not used in pharmacy [2].

Voltammetry is a method characterized by high sensitivity, accuracy, precision and broad linearity range, with relatively low-cost apparatus [3]. This technique was successfully used for the determination and assay of many drugs [4]. Chemically modified electrodes (CME's) are quite new approach in basic electrochemical experiments. The deposition of thin polymer film on the electrode surface endow the CME with desirable chemical or electrochemical properties.

In the current work poly-*N*-acetylaniline (PNAANI) glassy carbon (GCE) modified electrode was used for determination of fenamic acid in aqueous media.

Material and Methods

Electrochemical measurements were performed on a multipurpose Electrochemical Analyzer M161 with the electrode stand M164 (both MTM-ANKO, Poland). A three-electrode single-compartment cell was used for cyclic voltammetry. A chemically modified electrode was used as the working electrode, a platinum wire as the counter electrode, and a Ag/AgCl electrode as the reference electrode. The working electrode was prepared by electrodeposition of poly-*N*-acetylaniline film on the surface of GCE. A 2.0mM stock standard solution of sodium fenamate was prepared. A 8-ml solution containing an appropriate amount of fenamic acid and 0.3M Britton-Robinson buffer solution was transferred into the voltammetric cell. Cyclic voltammetry (CV) experiment was conducted by potential sweeping from -250 to 1000 mV.

Results

The effect of pH on fenamic acid oxidation was investigated in the pH range from 1.74 to 6.51. The peak current and peak potential are dependent on pH of Britton-Robinson buffer. The pH value of 3.30 was chosen as the supporting electrolyte. The relationship between the I_{max} for anodic peak current and the concentration of fenamic acid was examined by CV in the pH 3.30 in Britton-Robinson buffer at the scan rate of 100 mV/s (Figure 1).

- Anodic peak current increased linearly with the FA concentrations from 6.25×10^{-7} to 3.00×10^{-5} mol/L.
- A detection limit of 6.18×10^{-11} mol/L was obtained.

Discussion and conclusions

The oxidative reaction of fenamic acid can be used as an assay of the compound in the linear range of concentration 0.625μM to 30μM with detection limit of 61.8pM. Liner range is limited to 30μM by the low solubility of fenamic acid the supporting electrolyte.

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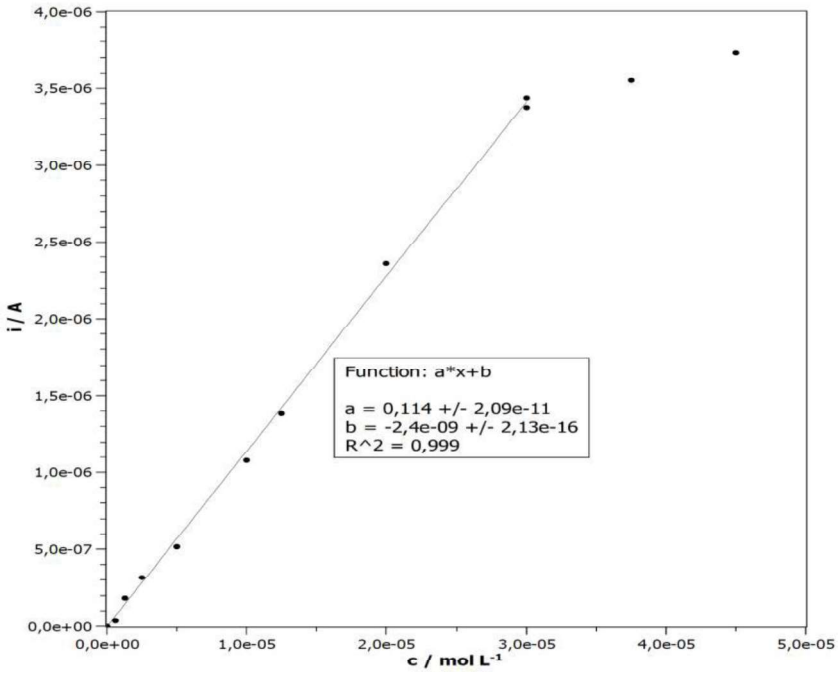

Figure 1 Calibration curve

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Preparation of nesfatin-1 – a novel multifunctional hormone peptide

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Background

Human nesfatin-1 is a 82-amino acid peptide hormone that was first discovered by Oh-I et al [1]. Nesfatin-1 is mainly involved in regulation of energy homeostasis and exhibits potent anorexigenic effect independent of leptin signalling when injected intracerebroventricularly in mice [2]. Nonetheless this hormone may be involved in many other important biological processes, such as regulation of anxiety and stress [3], reproduction [4], regulation of circadian rhythm and epilepsy [5,6]. Recent studies show that high levels of nesfatin-1 may be correlated with metastasis and poor prognosis of colorectal cancer [7], bladder cancer [8] or breast cancer [9]. Development of effective preparation methods of nesfatin-1 is thus important. Herein we present an efficient protocol for preparation of recombinant human nesfatin-1 from *E. coli* cells.

Material and Methods

Buffers: A (300 mM NaCl; 50 mM Na_2HPO_4, pH 7.0), B (buffer A supplemented with 35 mM imidazole, pH 7.0), C (buffer A supplemented with 200 mM imidazole, pH 7.0), D (150 mM NaCl; 20 mM Tris-HCl, pH 7.5). All agents were purchased from Roth beside NaCl which was purchased from Merck.

Affinity chromatography: Bacteria were lysed by sonication (8.5s bursts, 6.5s pause, 40 intervals) and 50 µl of DNase (10 mg/ml, Sigma) and RNase (10 mg/ml, Sigma) were added. Then the extract was centrifuged (12 000 ×G, 4°C, 50 min) and supernatant was collected. From then the experiment was conducted at 4 °C. Next 1 ml of Ni-NTA His•Bind® Resin (Merck) was equilibrated with 10 ml of buffer A and then incubated with the supernatant on the vertical shaker (12 RPM, 30 min). Supernatant was then loaded onto the column and the flow-through (FT1) was collected. Next the column was washed with 10 ml of buffer A and the wash fraction was collected. The column was rinsed with 5 ml of buffer B and 0.5 ml fractions were collected. Finally nesfatin-1 was eluted with 5 ml of buffer C and 0.5 ml fractions (E1-E10) were collected. Concentration of nesfatin-1 was estimated by measuring A280 (Nanodrop 2000c, Thermo Scientific).

HRV3C-tag digestion and nesfatin-1 purification: Fractions E2-E6 were pooled together and desalted to buffer A on the PD10 column (GE Healthcare) according to manufacturer's instructions. Next the concentration of nestatin-1 was estimated with A280 and the excitation coefficient of 0.45 $ml \times mg^{-1} \times cm^{-1}$ and HRV3C (Sino Biological) protease was added in the w/w ratio of 1/100 to remove the His-tag. The digestion was carried out on vertical shaker (12 RPM, 4°C, 12 h). Subsequently 200 µl of Ni-NTA resin was equilibrated with 4 ml of buffer A and incubated with the digestion solution on the vertical shaker (12 RPM, 4°C, 30 min). Then the solution was loaded onto the column and flow-through (FT2) was collected. Next FT2 was concentrated on the Amicon Ultra-4 (Merck) to about 500 µl volume. On each step the concertation of nesfatin-1 was estimated based on A280 measurement.

Size-exclusion chromatography: Further purification of nesfatin-1 was carried out on the Superdex 75 Increase 10/300 (GE Healthcare) column. First the column was equilibrated with buffer D and then the sample was injected. The fractions exhibiting nesfatin-1 presence were pooled and stored at -80°C until further evaluation.

Results

First stage of affinity chromatography yields about 2 mg of nesfatin-1 per 0.5 L of bacterial culture. At this stage the protein is slightly contaminated with bacterial proteins as proved by SDS-PAGE and Western-blot analysis. The subsequent steps yield about 1 mg of pure and homogenic nesfatin-1 sample as evidenced by SDS-PAGE, Western-blot and mass spectrometry analysis.

Discussion and conclusions

The protocol presented here allows for fast and efficient preparation of human nesfatin-1, which to our knowledge hasn't been described in the literature yet. We hope that this protocol will endow further research on this unique peptide.

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Correlations between selected markers associated with the red-ox status, with selected parameters of inflammation and glycaemic status in a mixed obese-non-obese population sample

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Background

As inflammation and increased body fat are coexistent with changes in the total antioxidative capacity (TAC), much research into antioxidants has been carried out, in order to determine the differences between obese and non-obese individuals, and associated alterations in basic clinical parameters, describing: glycaemic status, ongoing inflammation process and insulin resistance.

In this research, monotonic correlations between clinical parameters, and selected markers associated with the red-ox status, in plasma/serum, were checked for. The main purpose was to assess possible associations between the patterns in which oxidative stress affects antioxidative parameters, mainly – the concentration and/or activity of superoxide dismutase isozymes.

Material and Methods

The sample consisted of 94 individuals, of which: 50 were non-obese, and 40 – obese, as determined by a BMI cutoff (BMI ≥ 30). The male-to-female ratio in both non-obese and obese was moreless equal (21:29 and 24:20, accordingly).

The variables between which the correlations were tested are: concentration of superoxide dismutase isozymes (SODs): SOD1, SOD2, SOD3, total SOD activity, Cu,Zn-SOD activity, Mn-SOD activity, TAC, concentration of malondialdehyde (MDA), copper and zinc, cadmium, C-reactive protein (CRP), glucose and insulin concentration, HOMA-IR (insulin resistance parameter), age and BMI.

Results

The power of most of the significant correlations in this study was below ρ 0,5. Age negatively correlated with the activity of Cu,Zn-SOD, TAC, concentration of MDA, copper and cadmium. CRP negatively correlated with total SOD activity, positively – with TAC, MDA and copper concentration. Glucose concentration correlated positively with: TAC, MDA and zinc concentration, whereas insulin concentration correlated negatively with total SOD activity, and positively: with SOD1 concentration, TAC and MDA. HOMA-IR correlated with the same factors as insulin, except for MDA. BMI correlated negatively with: total SOD activity, Cu,Zn-SOD activity, and positively: with TAC, MDA and cadmium.

Interestingly, the concentration of SOD1 weakly and negatively correlated with the

concentration of SOD2, but positively: with Cu,Zn-SOD activity, TAC and zinc concentration. Both total SOD and Mn-SOD activity correlated negatively with copper concentration. TAC correlated with MDA.

Discussion and conclusions

No strong associations have been found between any of the described variables. The association between the concentration of zinc and the concentration of SOD1, and TAC might be due to the fact that zinc is an antioxidant, present in the active site of SOD1[1]. Decrease in SOD activity with an increase in age has been previously shown [2].

The positive correlations between TAC and: BMI, HOMA-IR, and CRP, glucose, insulin and MDA concentration may indicate that TAC is upregulated in strongly prooxidative conditions, presumably as means of adaptation aimed to restore red-ox homeostasis. The possibility of the organism to adapt to oxidative stress by upregulation of antioxidative capacity has been previously shown in skeletal muscles of non-insulin dependent type 2 diabetic men [3]. Negative correlations between CRP, insulin, HOMA-IR and BMI with total SOD activity may indicate inactivation caused by oxidative modifycations of SOD isozymes, or other processes ongoing processes, such as glycation [4, 5]. The lack of association between any of superoxide dismutase activities and TAC seems to support the thesis stating that TAC assays mostly measure antioxidative capacity of low molecular mass compounds [6].

The correlations found in this study seem logical. However, there are discrepancies between the correlations found in this study and these found in the literature. In a study by Lim et al., BMI correlated negatively with TAC [7].

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The role of apoA-I in regulation of paraoxonase-1 (PON1) activity

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Background

PON1 is a Ca^{2+}-dependent glycoprotein with a molecular mass of 43 kDa. PON1 are primary synthesized in the liver and then secreted into the bloodstream, where it circulates throughout the body in association with high density lipoproteins (HDL) particles [1]. PON1 can be evaluated by its different activities: paraxonase activity (against organophosphates such as paraoxon), arylesterase activity (against aromatic esters such as phenylacetate) and lactonase activity as a native activity of PON1 (against lactone-like structures elaborated by oxidized polyunsaturated fatty acids on lipoproteins) [2,3,4]. The role of PON1 is the protection from the oxidation

of HDL and LDL molecules. This enzyme, by destroying oxidized phospholipids, reduces the ability of oxidized LDL to induce monocyte chemotaxis and thus, limit the inflammatory processes in the vessel wall and inhibit the development of vascular and coronary diseases [1].

The antioxidative function is provided by the presence of HDL molecule in PON1 structure, which contains apoliprotein AI (apoAI) [5]. Aim of the paper was to analyze studies investigating influence of lipoprotein ApoA-I on PON1 activity

Material and Methods

Data was collected by anlyzing available articles which present results of studies related to PON1 and its association with apoA-I. Data were sought by computer-based searches from databases including PubMed, Google Scholar without language restriction. Search term combinations were keywords relating to the paraoxonase 1 (e.g., „paraoxonase", „PON", „PON1", „PON1 activity", „PON1 activity regulation") in combination with words related to apoA-I (e.g., „apoA-I", „PON1 and apoA-I"). One term was replaced each time until all possible combination mode were searched to avoid any missing literature. The titles and abstracts of potential articles were screened to determine their relevance. Chosen literature represent researches conducted between 1999 and 2019.

Results

A number of studies have demonstrated that HDL provides a vector that facilitates the secretion of the enzyme by liver, essentially by offering a hydrophobic harbor for the retained signal peptide of PON1 and coincidentally stabilizing the enzyme. The lipoprotein, such as apoA-I, also furnishes a hydrophobic environment that is important for PON1 function [6]. A positive correlation between apoA-I and PON1 levels *in vivo*, combined with its absent or low activities observed in apoA-I deficiencies (such as Tangier disease), suggested that apoA-I was required for the expression of PON1 as well as its binding to HDL [7]. It was shown positive associations between PON1 activities/concentrations and HDL-cholesterol and apolipoprotein A-I (apoA-I) concentrations [6]. Oda et al. revealed that apoA-I increases PON activity in a concentration-dependent manner, however it doesn't influence PON1 secretion from cells [8]. Sorenson et al. were noted that apoA-I is not required for the association of PON1 with phospholipid-containing vesicles, however serum PON1 activity is more stable in the presence of apoA-I and if apoA-I is absent, PON1 activity decreases rapidly [5, 7].

Discussion and conclusions

Serum concentration of lipoprotein apoA-I might affect activity of PON1. Although PON1 binding to lipoproteins does not require apoAI, its presence is necessary to maintain optimum activity and stability of the enzyme [8].

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Xenobiotics and estrogens as potential inducers of lipotoxicity

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Adipose tissue is not only responsible for storage of energy in form of lipids in human body (white adipose tissue) or generating body heat (brown adipose tissue), but also has been recognised in recent years as an endocrine gland [1]. Hormones produced by adipocytes are referred to as adipokines and play vital role in regulating fatty acids (FA) (in form of triacylglicerol (TG)) reservoirs in a cell [1, 2]. Main storage of FA is located in adipose tissue, but also a small reservoir of FA is present in almost every human cell, because FA are needed for example in process of phospholipid bilayers formation [2]. Adipokines are responsible for proper distribution of FA in body cells in cases of normal nutrition, overnutrition and malnutrition. When the homeostasis of adipokines is disrupted or when the adipose cells become unresponsive to adipokines, FA start to cumulate in nonadipose cells in larger amounts than they should, resulting in general steatosis, which leads to disfunction of nonadipose tissues [2]. Originally this state was described as lipotoxicty, but currently it is known as a more complex state. Too high amount of FA has destructive effects on glucose metabolism and it causes functional impairments in several metabolic pathways, both in adipose tissue and peripheral organs, like liver, heart and muscle. Additionally lipotoxicity plays an important role in insulin resistance and pancreatic beta cell dysfunction [3].

There are many potential factors, both endo- and exogenous, that can disturb organism hormonal balance, and thus may induce lipotoxicity. Different xenoestrogens, as an exogenous factors, or estrogens and its metabolites, as an endogenous agents, both are indicted as endocrine disruptors and potential inducers of lipotoxicity. A systematic review of the literature data relating to lipotoxicity in the aspect of xenobiotics and estrogens action was performed.

Estrogens and its metabolites act mainly by estrogen receptors, inducing DNA destruction, intracellular signaling pathway changes and/or oxidative stress [4]. These pathways can promote cells disturbances and they can also indirectly induce intracellular endocrine disturbances and probably promote the development of lipotoxicity. Xenobiotics may affect the level of adipokines in blood serum, adipocytes' response for adipokines leading to change of FA concentratios, therefore to insulin resistance, early stages of diabetes and steatosis of tissues. Moreover the metabolism of FA (usually on the oxidative way) takes alternate nonoxidative pathway which results in the rise of TG content. Products of further metabolism of TG may lead to dysfunction and even death of cells [2]. Free FA may also induce endoplasmic reticulum and mitochondrial stress [5,6]. Additionally xenoestrogens can mimic the action of estrogen, causing changes in cells similar to those induced by estrogens. One of the representatives of xenobiotics group with the potential of influencing hormonal homeostasis can be metalloestrogens, such as cadmium, nickel, antimony which are factors that can exacerbate lipotoxic condition [7, 8].

Investigation of phenomena of lipotoxicity caused by xenoestrogens and estrogens may elucidate yet unknown mechanisms of endocrine disruption connected especially with visceral adiposity and insulin resistance, which increase the cardiometabolic

risk and risk of metabolic syndrome development. Lipotoxicity seems to play a crucial role in the pathophysiology of these complex associations. Researching and understanding the possible mechanisms of interaction between the xenobiotics and estrogens mentioned above will provide valuable scientific information and guidance on the preventive actions of adverse effects of lipotoxicity.

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Does p53 mediate the upregulation of selected genes induced by cell treatment with actinomycin D and nutlin-3a?

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Background

Fourty years ago p53 was discovered as one of the first tumour suppressor genes. Studies carried out all over the world showed the multifaceted role of p53 in regulation of the response to cellular stress such as: DNA damage, hypoxia, oncogen activation or viral infection. Despite intensive research on *TP53* gene and its protein, there are still unidentified mechanisms concerning functioning of p53.

We have observed, that two substances, which stimulate p53 in different ways: actinomycin D and nutlin-3a, when acting simultaneously (A+N) induce synergistic activation of p53 in different cancer cell lines and in normal human fibroblasts. Probably, the synergy of these molecules results from the fact that actinomycin D stimulates phosphorylating of p53 by various kinases, whereas nutlin-3a helps the kinases in efficient phosphorylation of p53 by blocking the negative regulator of p53, the MDM2 protein. The analysis of transcriptome sequencing (RNA-Seq) of cancer cell lines exposed to A+N has revealed a significant increase in the expression of over 2000 genes, including expression of 500 genes up-regulated at least 10-fold. Based on our results and available binding site databases, we have found several genes not yet associated with p53 regulation: genes negatively regulating signalling through Wnt pathway: *DRAXIN*, *FRMD8*, genes connected with drug metabolism: *RETSAT* or genes with poorly understood function *FAM13C*, *KANK3* and *CTSH*.

Material and Methods

The cells in culture: A549 (lung cancer) have been treated with: actinomycin D and nutlin-3a. In order to confirm the hypothesis, the gene regulatory region of investigated genes with a potential p53 binding site has been cloned into pGL3-Basic reporter vector. Additionally, we have mutated the putative p53 binding site using site-directed *in vitro* mutagenesis system. The results of RNA-seq have been validated by Real-Time PCR. The selected genes, validated by qRT-PCR, will also be tested for their dependence on p53. We will compare their expression in control conditions or following A+N treatment in A549 cells with knocked-down p53 and in controls for knockdown.

Results

We have confirmed our hypothesis, that p53 affects the induction of *DRAXIN, RETSAT, FAM13C, KANK3, FRMD8, CTSH* following co-treatment with actinomycin D and nutlin-3a. The cloned promoters of investtigated genes contain *bona fide* p53 response element.

Discussion and conclusions

This recently identified new biological link between p53 and immunity, Wnt signalling pathway, drug metabolism and newly discovered p53-regulated genes deserves more detailed survey in further studies on these new functions of p53 tumor suppressor protein.

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This work has been supported by grants no. 2017/27/N/NZ5/01079 to BŁ-S from the National Science Centre, Poland (NCN).

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Anticancer activity of curcumin and wogonin in colon cancer cells

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Colon cancer affects a great number of people each year and is a second leading cause of cancer-related death in the world. Current oncotherapies are not fully effective due to high degree of resistance of colon cancer cells to cytostatic drugs. There is therefore a need for novel treatment options that could overcome or avoid drug resistance in this cancer. Combination therapy consisting of chemotherapeutic drugs and natural polyphenol compounds could improve the effectiveness of pharmacological treatment. Curcumin and wogonin are promising polyphenols for adjuvant cure. In this review, we provide an overview of their properties and mechanisms of action on colon cancer with special emphasis on theirs potential to improve the effectiveness of cytotoxic drug.

Curcumin, a bright yellow natural dye, is the component of turmeric, a common spice which found use in many cuisines all around

the world. Curcumin, chemically known as diferuloylmethane ($C_{21}H_{20}O_6$), is derived from rhizome of the *Curcuma longa* plant from the *Zingiberaceae* family. It was demonstrated by many published researches that curcumin possesses anti-toxic, anti-inflammatory, antioxidant and potentially chemotherapeutic properties. The anticancer effects of curcumin is associated by its inhibition of proliferation and angiogenesis in cancer cells and induction of apoptosis.

Wogonin, a monoflavonoid with chemical formula of $C_{16}H_{12}O_5$, is natural substance located in the *Scutellaria baicalensis* radix, the plant from the *Lamiaceae* family. The activity of the wogonin, supported by numerous studies, includes anticancer, anti-inflammatory, antioxidant and neuroprotective properties. In recent studies it was also showed that wogonin can display anxiolytic effects. The antitumor action of wogonin involves induction of apoptosis and cell differentiation and inhibition of several genes important for regulation of the cell cycle.

The chemopreventive activity of curcumin and wogonin in several animal tumor model systems strongly suggest their potential to improve cytostatic therapy in colon cancer. It has been shown that curcumin enhances the effects of irinotecan on colon cancer cells. Irinotecan is a key anticancer drug used for the treatment of metastatic colon cancer. Chemosensitization potential of wogonin in drug-resistant colon cancer has also been demonstrated. The combining of an anticancer drug with curcumin or wogonin may therefore improve cytotoxic effects of monotherapy and reduce undesirable side effects.

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Analysis of dendrimer-protein interactions and their implications on potential applications of dendrimers in nanomedicine

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Background

Dendrimers are three-dimensional nano-sized polymers. As these macromolecules are synthesised there is a controlled increase in the size, molecular weight, and number of surface groups with the increase of each increasing generation number. The chemical architecture of PAMAM dendrimers as well as their small sizes makes dendrimers an attractive proposition for research into their medical applications.[1]

PAMAM dendrimers are perfect candidates as carriers for the delivery of anticancer drugs due to them being highly soluble and having many chemically versatile surface groups. These allow for conjugation of anticancer drug molecules to develop dendrimer based drug delivery [1, 2].

The binding of protein to dendrimers can alter the structure, mobility, conformation and functional activity of the dendrimer with electrostatic forces playing a predominant role in the interactions between dendrimers and protein. In order to fully evaluate the potential of dendrimers in nanomedicine the impact of dendrimer-protein interactions must be understood.[3]

Material and Methods

The study used G5.5 PAMAM. The zeta potential of the dendrimer was calculated by the measurement of mobility from Electrophoresis, the measurements were taken at different values of pH, this was repeating using solutions prepared at differing ionic strengths.

The efficiency of G5.5 PAMAM adsorption on gold surface and the properties of the formed layer depending on the pH of dendrimer solution was also determined using a quartz microbalance with energy dissipation monitoring (QSense E1, Biolin Scientific) with a flow module. The adsorbed mass on the sensor for homogeneous, rigid films was calculated using the Sauerbrey model.

The size of dendrimer monomers dependant on varying pH was found in solution was measured using dynamic light scattering at differing ionic strengths.

The absorbance and wavelength of the G5.5 PAMAM dendrimer was measured for varying concentrations at constant ionic strength using UV-Vis. As well as for constant concentration while varying pH for solutions of different ionic strength.

Results

From the results of this experiment it was determined that the isoelectric point of G5.5 dendrimer was found to be at pH=5. It was noted that the initial zeta potential at natural pH (9.4-10.2) was negative and that as the ionic strength of the solution prepared increased the zeta potential at corresponding pH values increased, becoming less negative.

The adsorption of G5.5 dendrimer onto a gold surface under different conditions (pH, ionic strength and concentration) was investigated using Quartz Crystal Microbalance (QCM-D). Results from this experiment indicate that the maximum adsorption occurred at pH=5 corresponding to the isoelectric point of G5.5 dendrimer. It was found that on rinsing the gold surface with solution of the same ionic strength with a pH of 7.5 the dendrimer was seen to desorb from the gold surface.

The size of the of the monomers was found to range from 6.6nm to 8nm with there being an increase in size observed at pH=7.5.

The absorbance was found to be higher in solutions with lower ionic strength, with the wavelength being higher in solutions with higher ionic strength. It was noted that the that the absorbance and wavelength increased with increasing pH and with increasing concentration.

Discussion and conclusions

The G5.5 dendrimer was found to have properties that would present an attractive option as a drug carrier in nanomedicine. One barrier presented from the findings was the desorption of dendrimer when rinsed with solution of pH=7.5. The interactions of G5.5 PAMAM with bovine serum albumen and fibrinogen will be studied to determine what effects the dendrimer-protein interactions have on the secondary structure of the protein.

Acknowledgments:

This work was partially supported by project NCN OPUS 2016/23/B/02788, Erasmus+ UK GLASGOW 02.

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YM-1 as a modulator of HSP70 protein in chemotherapy combined with 5-fluorouracil

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Background

Recent studies have shown that molecular proteins, such as heat shock protein 70 (Hsp70) can be a potential target of anticancer therapy. Hsp70 is a stress inducible chaperon, and can be accumulated in cells in effect of various stressing factors provoking lethal conditions. In cancer cells Hsp70 is involved in oncogenesis and resistance to chemotherapy. Thus, the inhibition of Hsp70 seems to be a promising anticancer approach [1]. Here we proposed YM-1 molecule as an inhibitor of this protein. In multiple cancer lines, YM-1 was found to selectively target cancer cells over normal cells [2]. There was proved that YM-1 can preferentially bind to the ADP-bound form of HSP70 and therefore an inhibitory effect was observed [3].

The aim of our study was to compare the effects of YM-1 interaction with 5-fluorouracil (5-FU) against human colorectal cancer cells (LoVo).

Material and methods

Human adenocarcinoma cells (LoVo) were used as a model *in vitro*. 5-fluorouracil was implemented as a standard chemotherapeutic in colon cancer. YM-1 (2-[[3-Ethyl-5-(3-methyl-2(3H)-benzothiazolylidene)-4-oxo-2-thiazolidinylidene]methyl]-1-methylpyridinium chloride) was used as an inhibitor of Hsp70. Cell viability after exposition to 5-FU, YM-1 or combinations was evaluated by MTT assay after 24 and 48 hours. Hsp70 expression was semi-quantitatively determined by immunocytochemical method.

Results

We showed that YM-1 has anti-tumour activity in LoVo cells and that in combination with 5-FU after longer incubation (48h) resulted in synergistic effect. Immunocytochemical studies revealed an alternated expression of Hsp70 in colon cancer cells in particular after exposition to YM-1 or YM-1 combined chemotherapy.

Discussion

The available data indicate a pivotal role of the HSP70 in regulation of apoptotic cell death in cancer cells. Thus the modulation of chaperons appears an interesting approach in cancer treatment. Some authors indicated a unique YM-1 mechanism of action cause its ability to destabilize Hsp70–Bag3 activities, and finally to suppression of cancer-promoting signalling pathways [2]. There was shown that another inhibitor ADD70 applied in animal models of colon cancer and melanoma, showed promising effects on tumour size and growth and sensitized

cancer cells to chemotherapy [4]. Similarly, we have also proved a synergistic effect of YM-1 molecule with 5-FU in colon cancer, but further mechanism in this cancer type should be investigated.

Funding:
The research was supported by National Science Centre (Poland) within a framework of SONATA BIS 6 (2016/22/E/NZ5/00671; PI: J. Kulbacka).

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Cytotoxic effect of *Sculletariae baicalensis* derived flavonoid baicalein and ascorbyl palmitate in the treatment of Pancreatic Ductal Adenocarcinoma

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Background

Pancreatic ductal adenocarcinoma (PDAC) is among the most deadliest and the worst to diagnose and treatment type of cancer. It is predicted, that by 2030 PDAC will be the second leading cause of cancer-assosciated deaths in the USA, and it will surpass breast, colon and prostate cancers in that matter [1][2]. Currently available types of therapies based on gemcitabine and FOLFIRINOX are insufficient, and it leads to a need for develop new ways of therapy and drug development for PDAC. Liposomes are well known tools for enhancing bioavailabillity and pharmacokinetic of wide range of hydrophobic and hydrophillic drugs [3]. Due to enhanced permeabillity and retention effect (EPR), which in PDAC occurs in high rate, liposomes are potentially very good candidades in therapy of PDAC [4]. Baicalein (BAI) is one of the *Sculletariae baicalensis* flavonoid component, which have widespread of antiinflammatory, antiviral and anticancer properieties [5]. Ascorbyl palmitate (PalmAs) is lipid derivate of ascorbate, which is known for its potential anticancer activities against PDAC [6]. In this paper we examined the cytotoxic effect of BAI and PalmAS towards two pancreatic cell lines with correlation between cytotoxicity on control cell line NHDF. We have also combine these two drugs into single therapy. Lastly, we have prepared liposomal formulation of PalmAs and BAI with high entrapment effciency for enhanced delivery.

Material and Methods

All phospholipids were purchased in Lipoid, Germany. Cholesterol was donated by Hasco-Lek, Poland. Baicalein was purchased in Haoxuan Bio, China. Ascorbyl palmitate was purchased in Gonmisol, Spain. All organic and non-organic solvents was purchased in Chempur, Poland. PalmAs and BAI liposomes was prepared using solvent-evaporation metod with compound/ lipid weight ratio 1:10. Obtained thin lipid layer was dissolved in tert-buthanol and freeze-dried overnight. Obtained lipid cake was hydrated using 150 mM sodium chloride, and calibrated via extrusion. Size and polydispersity was measured using

Malvern ZetaSizer NanoZS. Concentration of PalmAS was measured via HPLC method. Concentration of BAI was determined by spectrophotometric methods. Cytotoxicity was determined using MTT assay with BAI dissolved in DMSO and PalmAS as liposomal formulation. Values of IC50 was calculated using GraphPad Prism 6.

Results

Size and polydispersity of PalmAs and BAI liposomes were suitable for intravenous application. Entrapment efficiency was nearly 100%. IC50 values for BAI in DMSO was: 29,83μM for BxPC-3 cells, and 29.84μM for AsPC-1 cells. For PalmAs in liposomes, these values were 124,73μM for BxPC-3 cells and 40,84μM for AsPC-1 cells. NHDF cell line was unaffected or affected in limited way only in the highest concentrations by those compounds. We have also observed doubled cytotoxicity by using these compounds combination on both cancer cell lines, with effect of this combination on NHDF cells for further investigation and determination.

Discussion and conclusions

Based on obtained data we believe that both of these compounds can be used in proposed liposomal therapy of PDAC. Both compounds carry potent cytotoxic effect against PDAC cell lines with limited cytoxicity against normal cell lines. Easy and efficient liposomal encapsulation process gives many prospects for future o potential of pharmaceutics suitable for intravenous administration because of their enhanced pharmacokinetics.

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Mechanisms of protective action of polyphenol extract Sea buckthorn *(Hippophae rhamnoides* L.) in relation to the erythrocyte membrane

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Background

Sea buckthorn extract is a rich source of nutrients, antioxidants and other components that can potentially exert protective role in living organisms. Attributed to it is an antioxidant and anti-inflammatory action, helpful in treatment of tumors and the cardiovascular, gastrointestinal and skin diseases [1-3]. The studies were designed to determine the polyphenolic composition and biological activity of extract of Sea buckthorn (*Hippophae rhamnoides* L.) in relation to erythrocyte membranes. In the study the erythrocyte was treated as an

example and model of the animal cell, and its membrane as a simple model of biological membrane, which is the first site of contact between physical agents and the body.

Material and Methods

Sea buckthorn extract was obtained from the Department of Fermentation and Cereals Technology, Wroclaw University of Environmental and Life Sciences. A detailed quantitative and qualitative analysis of extract was conducted, using the chromatographic (UPLC-DAD, UPLC-ESI-MS) and spectrophotometric (Folin-Ciocalteu) methods. The biological activity of the extract was investigated in relation to erythrocytes and isolated membranes of erythrocytes by using spectrophotometric and fluorimetric methods. Spectrophotometric method was used to determine the effect of the extract on the degree of haemolysis and osmotic resistance of erythrocytes. The antioxidant activity of Sea buckthorn extract towards erythrocyte membranes was determined with spectrophotometric and fluorimetric methods using two oxidation inducers: UVC radiation and AAPH.

Results

A total of 40 polyphenolic compounds found Sea buckthorn preparation were identified. The study confirmed high antioxidant activity of the polyphenols contained in Sea buckthorn extract, compared to that of Trolox®. The results of hemolytic investigations showed that Sea buckthorn extract does not induce hemolysis, which means there is no destructive action on the erythrocyte membrane. The osmotic resistance investigation of extract modified erythrocytes, using hypotonic NaCl solutions, did not show significant changes in the increase in osmotic resistance of erythrocytes.

Discussion and conclusions

Thanks to the rich polyphenolic composition and high antioxidant activity, Sea buckthorn extract protects the body against the harmful effects of free radicals. High antioxidant activity classifies the tested extracts as a valuable source of compounds that can find wide application in the prevention and treatment of many diseases arising as a result of disturbed oxidative processes in the body.

This study was supported by funds of the statutory activity of the Department of Physics and Biophysics of the Environmental and Life Sciences, University of Wrocław,

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Functionalization of curdlan gel for effective antibiotic bonding

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Background

Currently, various synthetic and natural polymers are used for regenerative medicine. One of natural polymers of high biomedical potential is curdlan which forms hydrogel of high strength and elasticity. Also, it exhibits high water-absorption and

retention capacity, therefore may be useful in fabrication of e.g. hydrogel dressing materials or other biomaterial resources (Cai and Zhang, 2017).

Coupling of therapeutical substances to curdlan matrix could increase its biological potential. However, the disadvantage of curdlan is the lack of active groups for modifications, such as amino and carboxyl groups which could enable binding of antibacterial or anti-inflammatory agents. Strategies used for curdlan functionalization include, among others, sulfonation, amination, oxidation, esterification or phosphorylation (Cai and Zhang, 2017; Zhang and Edgar, 2014) but they typically lead to curdlan solubilization and loss of beneficial physicochemical properties. Recently, catecholamine polymers were used for functionalization of different matrices (Lee et al., 2007). Therefore, catecholamine derivative was used to functionalize the curdlan matrix by formation of strongly adhesive ad-layer. Such functionalized matrix was used as a template for antibiotic (gentamicin) immobilization. Some of the properties of such produced drug-loaded hydrogel were tested, including antibacterial activity against three reference bacterial strains and drug release profile.

Material and Methods

8% (w/v) curdlan suspension gelled at 93°C was used as a matrix. Functionalization of polysaccharide matrix was performed by hydroxytyramine polymerization from 2 mg/ml (or 4 mg/ml) solution for 24 h at 25°C. Monomer (hydroxytyramine) was added to curdlan matrix before its gelation (sample 2-D-BG and 4-D-BG) or after (2-D-AG). Non-attached polymer was eluted from the matrix in DI water (as monitored by UV-VIS spectrophotometry). Gentamicin was immobilized on functionalized matrices during their incubation in 1 mg/ml aqueous drug solution with and without activation with 5% glutaraldehyde. Drug release from the matrices was performed by incubation of curdlan samples in PBS pH 7.4 at 37°C, with daily exchange of buffer. In collected samples, gentamicin was estimated after derivatization by phthaldialdehyde (Ginalska et al., 2004). Antibacterial activity of functionalized curdlan was evaluated by indirect method, in extracts collected in similar way as in drug release test (PBS was replaced by Mueller-Hinton Broth). The extracts were inoculated by *S. aureus* ATCC 25923, *S. epidermidis* ATCC 12228 and *E. coli* ATCC 25922 reference strains and allowed to grow at 37°C for 24h. Then the bacterial growth was estimated as optical density at 660 nm in Synergy H4 plate reader (Biotec, USA). The water uptake was evaluated during 72 hours incubation in water, as a function of wet weight increase.

Results

The catecholamine layer on matrix was regular, without noticeable precipitates and colour differences. Larger amount of gentamicin was immobilized to the curdlan sample which was modified by hydroxytyramine added before polysaccharide gelation (2-D-BG) in comparison with added after gelation (2-D-AG). 2-fold increase of amount of hydroxytyramine monomer for curdlan functionalization caused the slight (by approx. 25%) increase of drug immobilization. Activation with glutaraldehyde did not affect the binding capacity of the drug to functionalized curdlan matrix. The results of the experiment of gentamicin release to PBS solution indicated that the 4-D-BG sample the most effectively released the drug. Determination of the antibacterial properties of drug-loaded hydroxytyramine-functionalized curdlan samples showed that these matrices were able to protect the culture medium against bacterial infection for a long time. The weakest protection was observed against *E. coli* strain while against S. *epider-*

midis and *S. aureus* strains this protection lasted for a minimum 28 days. All samples absorbed water in 700-900 % of its initial weight.

Discussion and conclusions

Modification of curdlan matrix using polyhydroxytyramine allows efficient binding and release of the antibiotic and protects the matrix against bacterial infection. The matrix modified in this way can be used in future to create dressing materials for the treatment of postoperative wounds and to protect them against infection.

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Unravelling the mechanisms of the increased efficacy of electrochemotherapy after catechin incubation in pancreatic cancer

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Background

Catechins are green tea-derived polyphenols exerting the anticancer effects through a wide range of various mechanisms [1, 2]. In the previous study we have demonstrated that the co-treatment with catechin can firmly enhance the efficacy of electroporation (EP) with cisplatin in pancreatic cancer cells [3]. We proposed that the effect can be attributed to non-transcriptional mechanisms evoked in cancer cells such as the oxidative stress, the impairment of multidrug resistance proteins' function and finally, the direct impact on the membrane's permeability. Here, we examined if the catechin influences cell permeabilization following the delivery of short electric pulses.

Material and Methods

The impact of catechin on electropermeabilization was investigated using both theoretical and experimental approach. The molecular dynamics (MD) simulations were run and visualized with the GROMACS 2018.3, CHARMM-GUI and VMD software. We have examined the localization of catechin and its influence on the thickness of the membrane. Then, we have compared the threshold electric field enabling permeabilization with and without the catechin's presence. The model was verified experimentally by measuring the uptake of fluorescent dye YO-PRO-1 in pancreatic cancer cells in the presence of catechin, using a flow cytometry method. Finally, we have performed the electrochemotherapy with calcium ions in two cancer cell lines (EPP85 181 RDB, HPAFII) and control cell line (CHO-K1) incubated with catechin. We have measured cell viability with the MTS assay.

Results

MD study revealed that during the exposure to the electric field catechin does not easily cross the lipid bilayer but rather localizes at the border of the water-lipid phase (Fig. 1), affecting the membrane thickness. Notwithstanding the latter, catechin attachment had

no effect on lowering the threshold electric field enabling pore formation. This was confirmed experimentally as we did not observe increased uptake of YO-PRO-1 in the presence of catechin. Finally, unlike with the cytostatic compound [3] catechin did not increase the efficacy of the EP with calcium ions in pancreatic cancer cells.

Discussion and conclusions

Although much attention has been given to anticancer properties of catechins in recent years, data on their influence on bioelectrochemical processes remain limited. To our knowledge, this is the first study to suggest that the increased efficacy of electrochemotherapy *in vitro* following the catechin incubation is not correlated with the increased permeabilization. Another explanation of catechin action may be its interaction with the proteins responsible for drug resistance [4]. Therefore, next stage of the study is to investigate the relation between catechin incubation and the expression and function of these proteins in pancreatic cancer cells.

This research project is financed by the National Science Center (Poland) No. 2017/27/N/NZ3/01110 (to Olga Michel)

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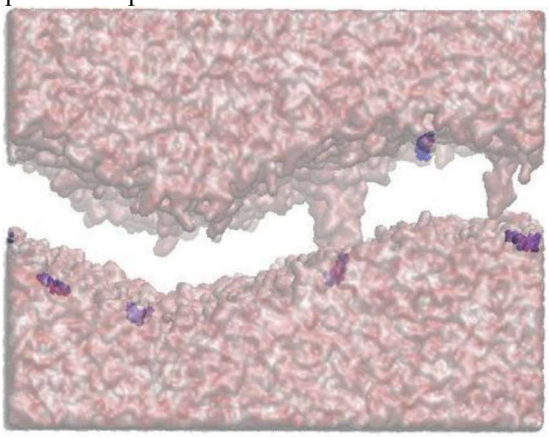

Fig. 1. The formation of pore in the plasma membrane under the influence of the electric pulse in the presence of catechin

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^{1}H nuclear magnetic resonance analysis of selected species of methanogenic archaea

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Background

NMR (nuclear magnetic resonance) has become one of the primary methods used for metabolomics studies in the last years. In addition to transcriptome and proteome – omics studies, metabolome analysis represents a third complementary approach for identifying the metabolic pathways. Metabolomics studies involve an analysis of microbial extracts (intra and extracellular). In microbial metabolomics NMR is used because it is nonselective, quantitative and reproducible method in metabolite quantitative and qualitative analysis [1].

Methane fermentation involves a complex series of metabolic reactions. During this processes, organic compounds are broken down into CO_2 and organic acids, which are further degraded by acetogenic bacteria into acetate, CO_2, and hydrogen. All these substrates are then preferentially utilized by methanogenic bacteria to produce methane. Sometimes the strains possess the reduced activity accompanied by decreased methane gas production. The reason of diminished methanogenesis is not known so far. Therefore the changes in the intracellular metabolites pathways of investigated microorganisms should be monitored and analysed [2].

The objective of this research is to present the initial results obtained by the ^{1}H NMR analysis of methanogenic archaea metabolome. Individual goals of the preliminary studies included: type and efficiency of extraction method, growth conditions influence, relationships between different species, metabolites identification.

Material and Methods

Biomass samples were obtained from NBRC (Japan). Lyophilized cells were homogenized and extracted with methanol/water solvent system. Then the samples were evaporated and dissolved in PBS buffer. All samples were investigated by use of ^{1}H NMR spectroscopy together with chemometric methods.

Results

Based on obtained NMR spectra, the PCA model was calculated with two principal components (PC), which revealed the natural grouping of the various bacteria strains. Three distinct groups of samples were formed: *Methanobacterium* cluster *M. acetivorans* cluster and *Methanosarcina* cluster (Fig. 1).

In addition to untargeted PCA analysis also supervised OPLS-DA analysis was performed according to internal parameters: medium substrates, additional reaction substrates and cultivation temperature.

The assignments of metabolite were performed for all found intracellular metabolites.

Discussion and conclusions

The obtained data by multivariate data analysis of spectra revealed taxonomical relationships between strains, while change in cultivation conditions has influence on the metabolic profile of *M. acetivorans*.

Further analysis of metabolomic relations between methanogenic archaea requires a broader set of species (and repetitions for each samples). More details regarding metabolic pathways (including higher number of identified compounds) would require wider metabolome analysis. Although, data were obtained on the limited number of samples, the results are auspicious. Due to the slow growth rates of individual archaea species, cultivation in chemostat conditions should be considered to facilitate the biomass collection.

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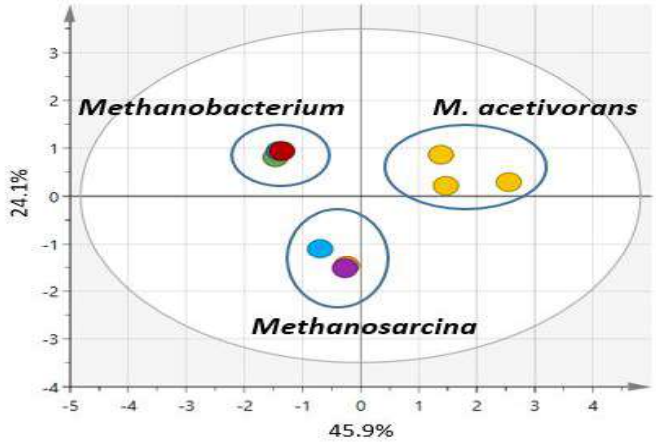

Fig. 1. PCA score plot of methanogenic archaea

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Differential effects of novel pyrimidine derivatives on hepatocarcinoma cells viability

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Background

Hepatocellular carcinoma (HCC) is the fifth (men) and ninth (women) most commonly occurring cancer in adult population. HCC-related mortality is still increasing in many countries as the majority of patients is present at an advanced stage of the disease [1]. New chemotherapeutic agents are constantly synthesized, many of which are heterocyclic compounds. Among many biological activities they present, the anticancer potential is one of our main interest.

A novel series of pyrimidine derivatives was synthesized. In their structure they contain an amino group at the position 4 and a hydroxymethyl or carboxyl group at the position 5. To the amino group various substituents were attached.

Our aim was to determine the antitumor activity of novel pyrimidine derivatives on human hepatoma cell line (HepaRG) by defining their half maximal inhibitory concentrations which inhibits biological function (IC50).

Material and Methods

For screening tests neutral red uptake assay was performed according to the protocol [2]. Briefly, compounds in appropriate concentrations were introduced into 24-hour cell culture. After the specified period of time, cells were incubated with a neutral red solution, followed by fixation with a 50% ethanol solution containing 1% glacial

acetic acid. The amount of uptaken neutral red was measured spectrophotometrically at 540 nm.

For flow cytometry experiments compounds with lowest antitumor concentrations selected after screening tests were analyzed. After incubation, cells were detached and fluorescently stained with propidium iodide (PI) and fluorescein diacetate (FDA) according to the modified protocol [3].

Results

Five pyrimidine derivatives with different substituents were tested. Amongst them three had a hydroxyl and two had a carboxyl group at the position 5. Compounds with hydroxymethyl group at the position 5 had lower IC50 than those with carboxyl group at the same place. Among the substituents at the position 4, tert-butylamine group was found to be the most effective cytotoxic agent (Tab.1).

Tab. 1. IC50 values obtained in screening tests for five pyrimidine derivatives. Substituents at the position 4 (s) are listed. Hydroxyl and carboxyl group at the position 5 are marked as (*) and (#), respectively.

	IC50 [μM]
s: amino group (*)	590
s: aminopropyl group (*)	600
s: tert-butylamine group (*)	80
s: aminopropyl group (#)	800
s: tert-butylamine group (#)	120

It is worth pointing out that cytotoxicity of pyrimidine derivatives concerns only cancer and not normal cells (viability of L929 cell line exceeded 90% in all tested concentration ranges).

Flow cytometry experiments provided information about the type of cell death.

Discussion and conclusions

The pyrimidine ring is a widely used structure in medicinal chemistry. It has been demonstrated that such structures show many biological activities i.e. antimicrobial, antiviral or antioxidant. Many used drugs with potential anticancer activity contain pyrimidine core [4]. Our newly synthesized pyrimidine derivatives show differential anticancer activity against hepatoma cell line. Based on IC50 values, the most promising compounds had tert-butylamine group at the position 4 with slightly better results for compound with hydroxyl group at the position 5. Further studies for precise molecular mechanisms of action and potential therapeutic usage should be undertaken.

The research was founded by Wroclaw Medical University (project number: SUB.D250.19.012)

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Genotoxic effects of resveratrol, celastrol and camptothecin in mono- and combined therapy in colon cancer cells lines

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Background
Colon cancer is the most prevalent and lethal malignancies. The major problem of successful treatment of this cancer is the existence of primary resistance and/or the acquisition of secondary resistance to currently available cytotoxic drugs. *In vitro* studies have found that some natural polyphenolic compounds, including resveratrol and celastrol, reduce chemo-resistance in different cancer cell lines. Here we investigated the genotoxic activity of both polyphenols in combinatory therapy with camptothecin, a key component of first- and second-line treatment regimens for metastatic colorectal cancer (CRC).

Material and Methods
The study was carried out using doxorubicin resistant LOVO/DX) and drug sensitive colon cancer cell line (LOVO). The original LOVO cells were derived from a fragment of a metastatic tumor nodule of human colon adenocarcinoma (Dukes'type c, grade IV, ATCC® CCL-229™). LOVO/DX cells were obtained by 3-month incubation of LOVO cells with low doses of doxorubicin. The genotoxicity was assessed by the means of FHA method (*Fast Halo Assay*). The cells were incubated for 24 hours in the presence of tested drugs in mono- and combined-therapy. The combination of 1, 5 and 10 μM of Celastrol or 5 and 10 μM of resveratrol with 10 μM of camptothecin were used.

Results
In monotherapy, an increase in the percentage of DNA damage was observed in LOVO cells for both polyphenols by 20-60% depending on the concentration tested. These effects were significantly stronger in LOVO/DX cells, i.e. 2-2.5 fold increase in DNA double strand breaks (DBSs) was noted. Combined treatment increased the genotoxic effect of celastrol when compared to camptothecin alone or celastrol alone. These effects were additive. In contrast, *c*ombined resveratrol and camptothecin induced only a very slight increase in the percenteage of DBSs.

Discussion and conclusions
The results indicate that both celastrol and resweratrol demonstrate significant genotoxic effect in colon cancer cells, especially in cells expressing high level of drug resistance. However, celastrol appears to be a better candidate for adjuvant treatment in this type of cancer due to its synergistic effects with cytotoxic drugs.

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This work was supported by the Wroclaw Medical University Grant No. SUB. D130.19.040.

High frequency effects on square wave electroporation efficiency

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Background

Electroporation is a phenomenon of increased biological cell membrane permeability facilitated by pulsed electric fields (PEF) [1]. From the perspective of pulse generation, electroporation induced electrotransfer of molecules depends on the pulse amplitude, duration and the number of pulses, however recently, alteration of the pulsing frequency gained increased interest and a new modality of electroporation protocols was proposed. It is based on high frequency (>0.5 MHz) unipolar submicrosecond pulse bursts, which enable manipulation of the electroporation efficiency using pulse repetition frequency as a sole parameter without change of the energy of the burst [2]–[6]. In this work, we have studied the capacitive charging of the cell membrane to predict permeabilization efficiency during high frequency electroporation.

Material and Methods

The finite element model of the biological cell was developed in COMSOL environment (COMSOL, Sweden) using an axisymmetric geometry. A free triangular mesh with 27931 domain elements and 511 boundary elements was formed [7].

During experiments, Chinese Hamster Ovary (CHO) cells CHO-K1 were used. For electroporation, the 0–3 kV, 60 A square wave 100 ns–1 ms pulse generator (VGTU, Vilnius, Lithuania) with a commercially available 1 mm gap electroporation cuvette (Biorad, Hercules, USA) was applied. Bursts of 5 kV/cm × 300 ns × 10 pulses have been generated at three repetition frequencies (1 Hz, 10 kHz and 1 MHz). Cell permeabilization efficiency was evaluated using propidium iodide (PI, Sigma-Aldrich) and flow cytometry (Amnis, Seattle, USA).

Results

After low (1 Hz) and medium repetition frequency (1 kHz) pulses electroporation efficiencies were comparable (20±5%), however 1 MHz protocol resulted in a dramatic increase of permeabilization efficiency (70±3%). The number of permeabilized cells increased solely due to the alteration of the pulsing frequency without any changes in the total energy of the burst, which was in agreement with the simulation model. It was shown that maintaining the transmembrane potential (TMP) above the threshold during the whole burst using MHz pulsing can effectively improve permeabilization of the cells. By means of generating high-frequency pulse bursts, it is possible to achieve a threshold repetition frequency when the discharging (TMP relaxation) time of the membrane will be higher than the delay time between the pulses, thus the TMP starts to accumulate throughout the burst. The simulation-predicted loss of the TMP accumulation phenomena in the low and medium frequency regions was also occurrent experimentally.

Conclusion

It was experimentally confirmed that the new modality of unipolar high frequency (MHz range) electroporation is a predominantly polarization-based phenomenon.

Acknowledgement

The research was funded by Research Council of Lithuania, Grant Nr. S-MIP-19-13.

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Shear-wave elastography as a new diagnostic tool in evaluation of masseter muscles stiffness

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Background

In modern high-end ultrasound machines, not only high resolution morphologic images, but also information on biomechanical properties of a tissue can be obtained. The ultrasound application named elastography allows quantifying of the elasticity of the musculoskeletal structures. In particular, shear-wave elastography is considered to be the most suitable type of ultrasound elastography for the musculoskeletal system. It is widely used for tendons, ligaments, and muscles [1-5].

Sonoelastography may be an optional objective method used in diagnostics of patients suffering from temporomandibular disorders (TMD), which is an umbrella term given to a variety of disease entities that involves the masticatory muscles, the temporomandibular joints and associated structures, or both. The etiology of temporomandibular disorders is not completely understood, and it is considered to be multifactorial according to the biopsychosocial model. Number of studies on prevalence, etiopathogenesis, diagnostics and management of temporomandibular disorders have been published in recent years.

Aim of this study is a standardization of sonoelastography measurement technique of masseter muscles on healthy subjects without TMD and determination of physiological values of stiffness of masseter muscles.

Material and Methods

Thirty healthy volunteers with full dentition or single tooth loss, without previous history of temporomandibular disorders and without any deviations from physiological function confirmed by the DC-TMD Axis I protocol were included (19 females and 11 males). Patients were examined using shear-wave elastography in prone position, with relaxed masticatory muscles. In total, the stiffness of 60 masseter muscles were measured using Aixplorer Supersonics Mach 30 machine with 18 MHz linear probe. Three measurements of each masseter muscle were performed. Descriptive statistics were used to analyse the collected data.

Results

The mean stiffness of the masseter muscles in healthy volunteers was 10.72±0.68 kPa in males (the mean age 37.2±2.9 years) and 10.58±1.25 kPa in females (the mean age

34.1±2.2 years). Optimal measurement technique of examination with probe positioned parallel to the long axis of muscle fibres and patient in prone position was established.

Discussion and conclusions

Shear-wave elastography proves to be useful to quantify masseter muscles stiffness. This method provides reliable and reproducible results. Elasticity of masticatory muscles is still a rather unexplored field of investtigation with a potential to improve the objective assessment of masticatory muscle disorders.

Acknowledgements

This work was financed by National Science Centre – grant no. PREL.B160. 18.007 (A. Olchowy).

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Klotho protein protects human cardiac myocytes from the damage during ischemia and reperfusion

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Background

Heart ischemia/reperfusion (I/R) injury causes an excessive formation of reactive oxygen species (ROS), reactive nitrogen species (RNS), degradation of contractile proteins by proteolytic enzymes and necrotic cell death of myocytes [1]. Klotho is a membrane-bound or soluble protein related to aging. Recent studies have proven production of Klotho in cardiomyocytes and its increased expression in stress-related heart injury [2].

The aim of this study was to examine an effect of Klotho protein on cell damage and degradation of contractile proteins in the cardiomyocytes subjected to I/R injury.

Material and Methods

Human cardiac myocytes (HCM) was maintained in aerobic conditions in the control group. In the study groups, HCM were subjected to *in vitro* chemical I/R (with sodium cyanide), in the presence or absence of recombinant human Klotho protein. Lactate dehydrogenase (LDH) activity served as a marker of cell injury. The level of oxidative and nitrative stress, and the degradation of contractile protein myosin light chain 1 (MLC1) were assessed.

Results

LDH activity was significantly higher ($p<0.05$) in cells subjected to I/R, compared to aerobic group. Incubation of HCM with Klotho protein significantly decreased ($p<0.05$) cell injury during I/R. Total ROS

and RNS activity was statistically higher ($p<0.05$) in I/R group in comparison to aerobically maintained cells. Klotho protein reduced the production of ROS/RNS ($p<0.05$) and enhanced total antioxidant capacity ($p<0.05$) in cells subjected to I/R. An expression of inducible nitric oxide synthase (iNOS) gene was significantly lower ($p<0.05$) and the level of nitrate/nitrite $(NO_x)^-$ was significantly higher ($p<0.05$) in myocytes from I/R group in comparison to aerobic group. The expression of iNOS gene negatively correlated with LDH level ($p<0.05$, $r= -0.78$), ROS/RNS activity ($p<0.05$, $r= -0.49$) and $(NO_x)^-$ level ($p<0.05$, $r= -0.51$), suggesting limited iNOS gene expression due to overproduction of nitrate/nitrite during injury. Administration of Klotho protein reduced $(NO_x)^-$ level ($p<0.05$) and increased expression of iNOS gene ($p<0.05$) in cells subjected to I/R. The amount of MLC1 in cell supernatants positively correlated with ROS/RNS activity ($p<0.05$, $r= 0.36$), which indicates degradation and release of MLC1 during I/R injury. The degradation of MLC1 was significantly lower ($p<0.05$) in HCM subjected to I/R in the presence of Klotho.

Discussion and conclusions

Reduction of oxidative and nitrative stress in cardiomyocytes injured by I/R suggests potential cardioprotective effect of Klotho protein. Klotho decreased cell damage and degradation of contractile proteins caused by I/R, thus Klotho may serve as a potential preventive/protective agent during ischemic injury of the heart.

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This work was supported by the National Science Centre, grant number UMO-2016/23/B/NZ3/03151.

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Fruits of interspecific hybrids grapevines as a source of polyphenolic compounds

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Background

Modern consumers are paying more and more attention to the presence of bioactive substances in food that can have a beneficial effect on the human body. Such substances include polyphenolic compounds, which are attributed to, among others anti-inflammatory, anti-virus or anti-cancer properties [1]. It has been confirmed that eating foods rich in polyphenolic compounds helps reduce the risk of developing civilization diseases (e.g. atherosclerosis, diabetes, cataracts or Alzheimer's disease) [2]. There is a growing interest in polyphenolic compounds present in various varieties of hybrid grapevines (interspecific hybrids), which are

grown in the temperate climate zone of the northern hemisphere.

The aim of the study was to assess the total content of phenolic compounds and the content of flavonoids in the fruits of selected hybrid grapevine varieties (interspecific hybrids) grown in ecological conditions in Poland.

Material and Methods

The research used fruit of 5 grapevines varieties: Michigan, Minesota, V 68021, Beta and Alwood, which were grown in the vicinity of Jarosław, in Poland. The total content of polyphenolic compounds fractions (skin, juice and pulp) was determined by colorimetric assay using the Folin-Ciocalteu reagent [3]. The total flavonoids content of the grapevine fruits was determined spectrophotometrically using aluminum chloride [3].

Results

Significant differences were observed in the content of polyphenolic compounds (including flavonoids) in individual parts of the grapevine fruit, as well as in particular grapevine varieties. The highest content of polyphenolic compounds in juice was recorded in the fruit of the Michigan variety (1,46 mg GA/ml), while the lowest - in the fruit of the Alwood variety (0,17 mg GA/ml). For example, in the grapevine skin of the Alwood variety, the flavonoid content was 26,4 mg quercetin/100 g dry matter, whereas for the Michigen variety it was 9,2 mg quercetin/100 g dry matter.

Discussion and conclusions

The obtained results suggest that the examined hybrid grapevine interspecific hybrids grown in Poland may be a rich source of phenolic compounds (including flavonoids). The research suggests that due to the content of bioactive compounds, the tested hybrid grapevine fruits can be considered as an important component of human diet with a potentially highly beneficial effect on human health.

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Antioxidant activity and reducing power of hybrid grapevines fruit grown in Poland

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Background

The special bioactive properties as well as the taste make the grapevine fruit (Vitis L.) widely used in the food industry. Due to the fact that there is a lot of highly processed food products on the food market, the interest in unprocessed natural food is growing among consumers in Poland. Changing dietary habits of consumers (looking for low-processed products with

high health-promoting quality) cause that the fruit of hybrid grapevine varieties play an increasingly important role in everyday nutrition [1].

The aim of the study was to assess the antioxidant and reducing activity and the total content of fruit of selected hybrid grapevine varieties (interspecific hybrids) grown in ecological conditions in Poland. The study examined the fruit of 5 grapevine varieties: Michigan, Minesota, V 68021, Beta and Alwood, which were cultivated in the vicinity of Jarosław, in Poland.

Material and Methods

The antioxidant activity of the grapevine fruit fraction (skin, juice and pulp) was determined spectrophotometrically using 2,2'-azinobis-3-ethylbenzothiazolin-6-sulfonic cation radical (ABTS) and using 2,2-diphenyl-1-picrylhydrazyl radical (DPPH)). The reducing properties of the grapevine fruit were also assessed based on the FRAP test [2-4].

Results

Significant differences in antioxidant activity were observed in individual parts of the grapevine fruit, as well as among varieties. The antioxidant activity of fresh grapevine fruit juice was of 0,069 μM Trx ml^{-1} for Alwood variety and 0,754 μM Trx ml^{-1} in Beta variety. Statistically, significantly higher antioxidant activity was observed in the grapevine varieties Beta, V 68021 and Michigan compared to the Minesota and Alwood varieties. Obtained research results suggest that in terms of health, the most valuable properties may have the Michigen variety, V 68021 and Beta.

Discussion and conclusions

The obtained research results suggest that in terms of bioactive and health-promoting properties, selected grapevine varieties (Michigen, V 68021 and Beta) can be considered as highly valuable products with potentially beneficial effects on the human body.

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Analysis of relationship between polymorphism rs11640851 in MT1A gene and MT, Cu and Zn concentration in the AP patients group

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Background

The acute pancreatitis (AP) is common illness among gastrointestinal diseases in many countries [1]. The pathogenesis of AP still has not been fully understood. It is believed that one of the major pathogenesis of AP is oxidative stress [2].

Metallothioneins (MTs) are cysteine rich low molecular mass (6–7 kDa) proteins. They plays a role as the antioxidant defenses. MTs have thiol groups of cysteine residues which have the highest affinity to zinc (Zn), copper (Cu) [3]. They take part in metalloregulatory processes and control cellular homeostasis of zinc/copper. MTs

are important for proliferation, differentiation and protection cells and tissues from free radicals [4].

In single nucleotide polymorphism (SNP) rs11640851 in MT1 gene is change from threonine to asparagine (from C to A nucleotide) [5]. This change is located in coding region in β domain. It could have an important influence on Cu/Zn balance and MT level in organism [5].

Aim of study

The aim of the project is to assess the impact of genetic polymorphism rs11640851 in MT1A gene on MT, Cu and Zn concentration in the blood of AP patients.

Material and Methods

Polymorphism in MT1A gene was studied by polymerase chain reaction (PCR) and restriction fragment length polymorphism analysis (PCR-RFLP). The results of the PCR product, were visualized by electrophoresis in agarose gel.

Measurements of metals (Cu and Zn) concentrations in serum will be determined by Flame Atomic Absorption Spectrometry method (FAAS).

The concentration of MT was measured in erythrocyte lysate and plasma using two-step direct ELISA method elaborated in our laboratory [6].

Results

It was observed decrease Zn concentration in the blood of non-smokers with CA genotype compare to non-smokers with AA and CC genotype. It was shown a decrease Zn concentration in the blood of smokers with CA and AA genotypes compare to smokers with CC genotype.

It was shown, that Cu/Zn ratio was increased in the group of smoking AP patients compare to non-smoking AP patients for CA, AA and CC genotypes.

Discussion

This study was shown, that SNP rs11640851 in MT1A gene could play important role in zinc homeostasis. It was confirmed that this genetic variation was associated with reduced intracellular zinc ion availability. Polymorphism in MT1A gene can contribute to decrease Zn concentration leading to imbalance in Cu/Zn ratio.

Conclusion

A decrease in zinc concentration in the blood of AP patients is associated with polymorphism rs11640851.

Abbreviations

AP: acute pancreatitis; MT: metallothionein, SNP: single nucleotide polymorphism, Cu: copper; Zn: zinc

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Evaluation of the diet of preschoolers from the Lower Silesian Province

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Background

Proper nutrition of children guarantees their proper mental, physical and social development. It consists in providing the body with all nutrients in appropriate quantities and proportions. From an early age, children should acquire healthy eating habits, and meals prepared in the kindergarten should be balanced and follow standards. Kindergarteners aged 4-6 years are particularly vulnerable to dietary mistakes, which can cause a lot of diseases later in life. Improper supply of energy and nutrients can contribute to disorders in the physical and mental development of the child's body. For this reason, it is very important to plan meals based on nutritional standards. The time that children spend in kindergarten is 6-7 hours per day. During this period they usually eat 3 meals, which should cover 75% of the whole day's energy and nutrient requirements. The aim of the study was to assess the energy and nutritional value of meals served in the kindergarten based on theoretical analysis of the menus using the computer programme Dietetician 2.

Material and Methods

The assessment was made by using the quantitative and qualitative methods, comparing the value of energy and selected nutrients (proteins, fats, carbohydrates, vitamins: A, B_1, B_2, C, E, mineral ingredients: Ca, Fe) in the analyzed menus (ten for each season of the year: spring, summer, autumn and winter) with the demanded standards for children aged 4-6 years [1].

Results

The qualitative evaluation of the menus showed that the breaks between meals were not appropriate, the meals were prepared using various techniques, salt consumption was reduced by replacing it with herbs, seasonal vegetables and fruit were used in the dishes. In spring, summer, and winter, the energy value of the food ration was adequate and within the limits of the standards, while in autumn it was above the standards. The quantitative analysis showed an adequate protein intake in relation to the recommended standards. Fat content, only in autumn, was within the range of accepted standards; in the remaining seasons of the year it was too low. Carbohydrate content was too high in relation to the recommended standards in all seasons of the year, except for winter. The examined menus did not cover the demand for selected mineral components. The content of calcium and iron in each season was too low in relation to the recommendations. The results of the analysis of menus showed that vitamins A, B_2 and C, in all seasons of the year, exceeded the accepted standards. The supply of vitamin E in the analyzed menus was at a correct level, in all seasons of the year. The analyzed menus covered the demand for vitamin B_1 only in summer. In the remaining seasons of the year the supply of this ingredient was too high in relation to the standards.

Discussion and conclusions

The supply of carbohydrates exceeded norms in all seasons of the year except winter. Too high carbohydrate level in children's diets can contribute to overweight and obesity. The content of vitamin B1 ranged from 0.48 mg in summer to 0.73 mg in spring and only in the summer the supply of this vitamin was within the range of accepted standards and in the remainings, it was too high. Since thiamine has a limited ability to be absorbed from the gastrointestinal tract into the human body, there is no risk of adverse effects of its excessive consumption. Excessive consumption was found for vitamins C, B2, and A. In case of excessive intake of ascorbic acid, there is no clear evidence of adverse effects; however, attention should be paid to the risk of gastrointestinal disorders. The high amount of vitamin B2 in the studied menus was the result of frequent offering to children a meat and meat products (ham, and sausages). In the analyzed food rations, the content of vitamin A exceeded the norms almost twice. Excess vitamin A in the body may be manifested by headache, increased excitability, vomiting, and skin changes. Consuming this vitamin in large quantities leads to a loss of calcium from the bones, and thus to a decrease in bone mineral density, which results in osteoporosis. The calcium and iron content below the norms did not differ significantly depending on the season of the year. The low amount of calcium in the diet is unfavourable, especially in children, in whom chronic calcium deficiency may cause rickets. With prolonged calcium deficiency in the diet, there is a decrease in peak bone mass, which in turn can result in osteopenia. To increase the amount of calcium in the preschoolers' diet, more milk and dairy products (cheese, cottage cheese, and natural yogurt) should be given.

The results of the study indicate the need for intensive education about the proper meal composition among employees of educational institutions (kindergartens).

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Box Diet – Can You Rely on It?

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Background

In recent years the pace of human life has increased significantly. Usually, overworked people do not have time to prepare five meals a day by themselves and use the services of catering companies offering ready-made meals that are delivered directly to the client's place of work or residence in the form of a box. Consumers ordering diets through dietary catering companies are convinced that they are in the hands of professionals. Usually, on the websites of companies offering boxed diets, you can find information that the diets are tasty and healthy. The word "healthy" should guarantee that the diets are arranged in accordance with the rules of menu arrangement and balanced appropriately.

The aim of the study was to evaluate the diet of individual customers on the basis of the standard diet developed by one of the companies (the company was randomly chosen) engaged in dietary catering, based in the Lower Silesia Province.

Material and Methods
This evaluation was carried out on the basis of menus prepared for ten days. For this purpose, the Diet 6D programme, developed by the Institute of Food and Nutrition in Warsaw, was used. The energy value and content of selected nutrients in each of the ten menus was calculated.

Results
The analysis of the diet catering menus showed that they were not composed correctly. The shares of basic nutrients in the standard dietary menus adopted by the catering company did not comply with the recommendations for a rational diet. Too low a percentage of carbohydrates and too high a percentage of fats were found. The analyzed menus also contained too low amounts of potassium and iron in comparison with the standards. The content of protein, salt, sodium, vitamins A, C, and B_2 exceeded the standards. The amount of dietary fiber, magnesium, and thiamine in the diet was in accordance with the standards. The advantage of the served meals was the fact that every day customers were offered a variety of meals rich in vegetables and fruit.

Discussion and conclusions
It should be emphasized that on its website the company described its diets as "healthy" while, at the same time, offered rations that were not properly balanced and arranged in accordance with the rules of food preparation. It is therefore necessary to make consumers aware that the diet catering service does not always meet their expectations.

Efficacy of biofilm eradication of *Staphylococcus aureus* strains isolated from wounds by the antimicrobials commonly applied to treat wound infections

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Background
The *Staphylococcus aureus* highly cross-linked biofilm matrix is a barrier for antibacterial compounds, contributing to persistent prevalence of this bacterium in wound infections [1]. The antimicrobial agent for treatment of wound biofilm should be selected with regard to the microbial species being etiological factor of infection, its ability to form biofilm, resistance to antiseptics, antibiotics and possible side effects [2, 3]. The aim of this study was to compare the efficacy of antibacterial agents, commonly used to treat wound infections, in eradication of *Staphylococcus aureus* biofilm isolated from wounds.

Material and Methods
11 strains of *Staphylococcus aureus* (including reference ATCC 6538 and ATCC 33591 and 9 clinical strains) were used for experimental purposes. The gentamicin antibiogram was performed in accordance with EUCAST guideline from 2017. The antibiofilm activity of the

antibacterial agents was examined using Minimum Biofilm Eradication Concentration (MBEC) method. The following antimicrobial agents were scrutinized: polyhexamethylene biguanide with betaine PHMB (0,1%), povidone iodine PVP-I (7,5%), sodium hypochlorite/hypochlorous acid solution NaClO/HClO (0,01%) and gentamicin GENT (0,1%). The drop of biofilms' metabolic activity followed by exposure on antimicrobial agents was measured using the Richards method. Statistical analysis of results obtained was performed by Statistica version 13, using Shapiro-Wilk test, Levene's test and U-test with p-value 0,05. All tests were performed in 2 repetitions and 3 replications.

Results

While antibiogram results revealed that all tested strains were susceptible to gentamicin, application of 0,1% of this antibiotic did not lead to complete biofilm eradication in case of 10/11 strains. Also 6/11 biofilm-forming strains proved to be resistant to 0,01% HOCL/NaOCl solution. Therefore, these compounds were not taken into account in statistical analysis. The average dilution of PHMB-based and PVP-I-based products, which enabled the complete biofilm eradication, was 35% and 22%, respectively. It translated into 0,36 g/L of PHMB and 17,05 g/L of PVP-I active substances. Both differences were statistically significant.

Discussion and conclusions

The tested agents used in the treatment of wound infections have shown various efficacy against staphylococcal biofilm. Gentamycin and hypochlorite compounds showed the lowest effectiveness. While strains were sensitive to gentamicin (according to antibiogram-based methodology), the biofilm formed by the same strains displayed high resistance against gentamycin antibiotic. Our results stay in line with data provided by another research team [4].

Twice-diluted NaOCl/HOCl agent showed no antibiofilm activity, however other researchers shown that higher concentrations of hypochlorites may display such feature. antimicrobial. Nevertheless, such concentrations are also harmful to fibroblasts cells, responsible for wound healing [5]. Substances containing polyhexamethylene biguanide with betaine and povidone iodine were the most effective. Even low concentrations of agents allowed to eliminate staphylococcal biofilm. However the application of iodine povidone has certain limitations. It cannot be administered to patients with hypersensitivity to iodine, hyperthyroidism or Duhring syndrome. The broad antimicrobial spectrum of PHMB and rare side effects indicate on possible use of PHMB-containing agents as a first-choice agent in the treatment of wound infections [2, 3].

Research was performed by means of Wroclaw Medical University statutory research SUB.D230.20.002 and STM. D230.20.127

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Isobavachalcone increases doxorubicin accumulation in resistant colorectal cancer cells HT29/Dx

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Background

According to the World Health Organization, cancer is one of the world's largest health problems. It is estimated that almost 10 million people died prematurely as a result of cancer in 2017. One of the major obstacle in the successful therapy of neoplastic diseases is multidrug resistance (MDR). Such phenomenon may be the result of several mechanisms involving various aspects of cell biology. However, a pivotal role in the insensitivity of cancer cells to drug is played by ABC pumps. These proteins are located in the membranes and decrease drug concentration within the cell. Among ABC transporters, P-glycoprotein (P-gp) is the best known mediator of MDR phenotype and its inhibition could improve cancer treatment. Natural products are very important source of promising leads for the development of novel chemosensitizers [1]. In our work we focused on the activity of isobavachalcone – phytochemical derived from Psoralea corylifolia. Previous studies shown that antiproliferative effects of isobavachalcone against human cancer cells may be related to its inhibition of Akt signaling [2].

Material and Methods

Cell based assays were performed on colorectal cancer cells HT29 and MDCK cells (Madin-Darby canine kidney) and their sublines characterized by P-gp overexpression (HT29/Dx and MDCK-MDR1). Cytotoxic activity was studied using sulphorhodamine (SRB) method. In order to measure doxorubicin accumulation fluorescence microscopy was applied. The interaction of isobavachalcone with lipid bilayer was investigated by differential scanning microcalorimetry (DSC).

Results

We did not observe antiproliferative activity of isobavachalcone in HT29 and HT29/Dx. However, using MDCK model cells we found that this compound was a substrate for P-gp. These results were also confirmed in the experiments with P-gp inhibitor. The application of doxorubicin – drug that is effectively removed from the cell by P-gp pump – together with isobavachalcone allowed for the increased uptake of the cytostatic by HT29/Dx cells. Also, our studies demonstrated that isobavachalcone changed biophysical properties of lipid bilayer.

Discussion and conclusions

The results indicated that isobavachalcone was the substrate of P-gp and had ability to increase doxorubicin accumulation in drug-resistant cells. Thus, the chalcone might be a candidate for MDR-reversing agent. Our work suggested that MDR reversing potential of isobavachalcone could be the result not only of its interaction with P-gp but also the ability to modify the lipid bilayer – the natural environment of ABC transporters.

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What is the influence of ketogenic diet on the state of gut microbiota?

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Abstract

The ketogenic diet (KD), whose main principles are high supply of fat and low supply of carbohydrates, has become increasingly popular in recent years [1, 2]. Initially, KD was used to support the therapy of illnesses with a neurological basis, but nowadays it is also increasingly often used as a restriction and alternative diet in sportsmen [1-6, 8]. Because KD can lead to undesirable consequences, the International Ketogenic Diet Study Group issued recommendations indicating groups of patients that can follow this nutritional plan. These recommendations comply with the directives of the National Centre for Nutritional Education [3, 9].

The most recent studies (in the last 5 years, PubMed, Scopus) [6, 7, 10] reveal that KD influences the composition of gut microbiota. KD reduces the diversity of gut microflora while causing an increase in positive bacteria instead of pro-inflammatory bacteria [6, 7]. Studies that were initially conducted on animals have now been confirmed in humans [10]. The studies revealed a significant decrease in the colonisation of intestines by Bacteroidetes spp, and an increase in Firmicutes spp. and Proteobacteria spp..

Microbiome diversity was observed, e.g. in infants with drug-resistant epilepsy where KD was applied. We now know that gut microbiome plays a crucial role in maintaining the integrity of gut barrier. It modulates energy metabolism and prevents inflammations observed in numerous illnesses [11, 12, 16].

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Effect of acrylamide supplementation on the population of vasoactive intestinal peptide (VIP)- immunoreactive neurons in the porcine small intestine

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Background
High levels of acrylamide have been shown in food products manufactured and processed at high temperatures (such as chips, cornflakes or coffee) [1]. Although the gastrointestinal tract is the main absorption route of acrylamide [2], little is known about its effect on the enteric nervous system (ENS) neurons. The aim of present study was to elucidate the impact of supplementation of low and high doses of acrylamide on the population of vasoactive intestinal peptide (VIP)- immunoreactive neurons in the porcine small intestine.

Material and Methods
The study was performed on 15 gilts divided into 3 groups: C group- the animals were administered empty gelatine capsules; LD group- the animals were administrated tolerable daily intake (TDI) dose (0.5 µg/kg b.w./day) of acrylamide capsules and HD group- the animals were administrated high-dose (5 µg/kg b.w./day) acrylamide capsules for 28 days. After supplementation period all animals were euthanized and fragments of duodenum, jejunum and ileum were collected and fixed. The frozen sections (14µm thick) from the collected intestines samples were then processed with the double immunofluorescent staining method using protein gene product 9.5 (PGP 9.5; mouse monoclonal, Biogenesis, cat. No. 7863-2004, working dilution 1:1000, used here as a pan neuronal marker) and VIP (rabbit polyclonal; Biomol, cat. No. VA1285; working dilution 1:6000) antibody as well as appropriate secondary antibody (Alexa Fluor 488 and 546).

Results
Acrylamide supplementation affected immunohistochemical characteristics of ENS neurons in the porcine small intestine. The increase in the number of neurons showing immunoreactivity towards VIP was noted in all fragments studied. The most remarkable changes was noted in the inner submucous plexus (ISP), in which a sta-

tistically important increase was observed in both experimental group (LD and HD) in all parts of intestine (duodenum: from 10.96 ± 0.65% in C group to 14.21 ± 1.03 % in LD group and to 20.65 ± 1.23 % in HD group; jejunum: from 14.02 ± 0.64 % to 25.27 ± 0.94 % and to 29.92 ± 1.32 %; ileum: from 14.38 ± 0.98% to 15.09 ± 0.91% and 22.62 ± 1.52 %, respectively). In the outer submucous plexus (OSP) statistically important increase was noted in animals receiving low and high doses of acrylamide only in the ileum (from 11.90 ± 0.29% to 14.93 ± 0.26 % and to 15.45 ± 0.69 %), whereas in duodenum and jejunum only in the HD group increase was important (from 11.00 ± 0.08% to 14.96 ± 0.45 % and from 14.54 ± 0.33% to 18.47 ± 0.37%). Similarly, in the myenteric plexus (MP) an increase in number of neurons immunoreactive to VIP was noted in both LD and HD group only in ileum (from 13.40 ± 1.38% in C group to 20.68 ± 0.81 % in LD group and to 28.7 ± 1.21% in HD group). In the duodenum and jejunum in HD group an increase was also significant (from 12.14 ± 0.31% to 16.71 ±1.63% and from 11.70 ± 0.33% to 20.14 ± 1.27%).

Discussion and conclusions

The recorded changes revealed that even the low doses of acrylamide influence the nervous structures located in the porcine small intestine wall. This may result from the neurotoxicity of acrylamide or from the response of the ENS to the acrylamide-induced inflammation and well corelate with previous study confirmed neurotoxic properties of acrylamide [3]. VIP is known to be an important neuroprotective factor, which stimulates mitosis within the astrocytes, supports neuronal differentiation of embryonic stem cells, and increases neuronal survival under various pathological factors [4]. Obtained results suggest that VIP plays an important role in protecting the gastrointestinal tract during acrylamide intoxication.

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Heat-induced changes in nuclear proteins associated with lamin in the *Drosophila melanogaster* model system

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Background

One of the best examined extracellular stressors is heat-shock induction. Cells in response to increased temperature have developed an evolutionarily conserved process- heat shock response (HSR). During HSR the heat shock transcription factor (HSF) binds to the promoters of hsp (heat shock proteins) genes resulting in activation of heat-inducible genes and a global downregulation of transcription. Moreover, it has been found that after heat shock induction the decondensation of chromatin occurs [1].

Changes in interaction between chromatin and protein after heat shock were also observed with major karyoskeletal proteins involved in chromatin organization– lamin. It belongs to V-type intermediate filaments

exerting structural and regulatory functions in the cell nucleus [2]. Our hypothesis is that lamins, together with topoisomerase II (Top2 is an enzyme required for DNA regulations) may play a key role in chromatin remodeling during HSR.

For our studies, we chose *Drosophila melanogaster* as a model system due to the presence of only two lamin genes – B-type (lam Dm) and A-type (lam C) and a single isoform of HSF, which makes it a definitely simpler model than vertebrates. In this study, we focused on investigating differences between normal and heat shock condition with regard to changes in protein complexes associated with lamin Dm together with post-translational modifycations which may be crucial in processes occurred during HSR.

Material and Methods

All experiments were performed on *D. melanogaster* embryonic cell line – Kc. Cells were maintained in suspension culture (in Schneider`s Drosophila Medium from Gibco with 10% FBS and 1% antibiotics) at 23°C as normal conditions. To induce the heat shock cells were incubated at 37°C for 1 h before further experiments. To identify proteins interacting with lamin 1% PFA cross-linking (10 min, RT) followed by co-immunoprecipitation (co-IP) under denaturing conditions (based on the protocol from ThermoFisher dedicated to Pierce Protein A/G Magnetic Beads). Samples after co-IP were next digest by FASP method, tryptic peptides were analyzed by tandem mass spectrometry analysis (LC-MS/MS). MS/MS data were processed using the Mascot searching engine (UniProt *Drosophila* database combined with The common Repository of Adventitious Proteins, cRAP).

Results

We aimed to confirm the interaction between lamin Dm and topoisomerase II in both, normal and heat shock conditions. We observed extreme change in the number of proteins identified in MS after heat shock (almost 70 more interactors identified in comparison to control). After the classification of identified proteins, we observed changes in clusters in both groups based on protein functions. In HS samples we observed an increased number of proteins involved in DNA/RNA binding. Based on the quantitative analysis we showed about 30% decreased of lamC identifiers (the best-known interactor of lamDm, q-value= 0,03), 30% increase of Top2 identification after hs (but the result is ns).

Discussion and conclusions

Previous experiments suggest that lamin and topoisomerase II are involved in the regulation of transcription during heat shock induction and moreover they interact directly with chromatin. We showed the interaction between them and along with other protein identifications from co-IP experiments its confirm us in this belief. To determine whether the interaction is direct or indirect (through chromatin) further experiments have to be performed (co-IP with nucleic acid digestion). Changes in lamin- interacting proteome may be the result of the re-localization of lamin Dm after induction of heat shock or might be the effect of different phosphorylation rates in both conditions. Observed protein pattern of interactors with lamin Dm after heat shock induction leads us to conclude that lamins may play a role in the epigenetic shutdown of transcription after heat shock-induced together with other components of a protein complex involved.

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Interaction of rhodium(III) cytostatic complex with red blood cells and cell membranes

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Background

The interest in the antitumor activity of metal compounds was caused by B. Rosenberg's discovery of the cytostatic effect of cis-diaminodichloroplatin (II). Since then, many new metal complexes have been studied for their potential use in medicine. Very promising cytostatic properties are exhibited by Co, Rh, Ir, and Pd coordination compounds with nitrogen and phosphine ligands [1, 2]. Our initial/previous tests for the anti-tumor activity of the rhodium (III) complex with tris (2-carboxyethyl) phosphine (in short – RhTCEP) have shown that it is cytotoxic to many cell lines, e.g. SK-mel (malignant melanoma), SH-4 (melanotic melanoma), Colo-829 (malignant melanoma) and C-32 (amelanotic melanoma) [work in preparation]. To explain the mechanism of biological activity of RhTCEP a number of further studies should be performed, including interactions with biological membranes – many chemical compounds exhibit biological activity through their direct or indirect effect on protein structures and cell membranes.

The overall purpose of the research task was to investigate the effect of RhTCEP on red blood cells and membranes. This task has been divided into the following stages:

1. Examine the hemolytic activity/toxicity of RhTCEP.

2. Check the effect of RhTCEP on the properties of the erythrocyte's membrane.

3. Check the influence of RhTCEP on the structure and properties of lipid model membranes (liposomes).

Material and Methods

The analysis was conducted on erythrocytes (porcine), erythrocyte lipid-protein membranes (RBCM) and lipid membranes (liposomes) without (SUV) and with cholesterol (SUV/chol). The research was carried out using several complementary methods. The spectrometric method was used to perform the first task. The second task was performed based on an optical microscope (analysis of erythrocyte shapes), fluorescence spectroscopy with the use of fluorescent probes as markers of lipid membranes and spectroscopy of attenuated total infrared reflection (Fourier transform infrared attenuated total reflectance, FTIR-ATR). Fluorimetric method was also used to measure fluorescence anisotropy and the degree of ordering of lipid bilayers of the model membrane (task 3). All measurements were performed at 37 °C.

Results

Hemolysis is a process during which hemoglobin flows out of the cell as a result of damage to the membrane or an increase in its permeability. Tests conducted over a wide range of concentrations from 10 to 200 μM of the test compound showed that RhTCEP does not cause membrane damage. It probably interacts with the hydrophilic area of the outer monolayer of the membrane, as evidenced by the change in the shape of 60 μM RhTCEP modified blood cells.

Infrared spectroscopy provided a lot of information about the structure and intermolecular interactions in the protein-

lipid membrane. Characteristic frequency bands of the protein and lipid components were analyzed in the IR spectra, which can be divided into the spectral regions that originate from the molecular vibrations of the hydrocarbon tail, the interface region and the head group. We identified the RBCM frequency bands for individual membrane components: methyl groups and methylene hydrocarbon chains, carbonyl, phosphate and choline groups of lipids and also the amide I, II and III bands. The RhTCEP does not change the fluidity of RBCM in the hydrophobic region of the lipid bilayer. However, slight changes are visible in the vibration bands derived from the phosphate group ($\nu_{as}PO_2^-$ and $\nu_sPO_2^-$) of the polar part of the phospholipid membranes. The $\nu_{as}PO_2^-$ vibration band shows high sensitivity to environmental polarity and the possibility of interaction via hydrogen bonds. Changes in this area indicate that RhTCEP interacts mainly with the polar part of the lipid membrane. This is also evidenced by the results of measurements of anisotropy and of generalized polarization factor (GP).

Conclusions

In summary: we can conclude that RhTCEP in the studied concentration range is not hemolytic active. It does not disturb the erythrocyte membrane, whereas it slightly affects its properties.

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This work was sponsored by the statutory activities of the Department of Physics and Biophysics of Wrocław University of Environmental and Life Sciences.

Impact naringenin and its derivatives on p53 and Bcl2/Bax expression in human colon adenocarcinoma cells HT29

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Background

Naringenin is a natural flavonon present in many plants, especially in citrus fruits. It has been shown as an potential anticancer agent [1]. However, the molecular mechanism of its action is still unclear. Some studies indicate that naringenin may induce an internal pathway of apoptosis, by regulation of p53, caspase 9 and Bcl-2 family members expression [2]. On the other hand its effecttiveness is limited by low stability and relatively high IC50 vales. For these reasons, new naringenin derivatives are being sought to improve the anticancer therapy. Previously, our group has shown that 7,4-di-O-butylnaringenin and its oxime have stronger cytotoxic effect on human colon adenocarcinoma cell lines than naringenin [3].

Material and Methods
We used HT29 colorectal adenocarcinoma cells for our research. First we induced apoptosis by add Naryngenin and it's derivatives into the cells, and then studied the expression of p53, Blc-2 and Bax thanks to Real-Time PCR and Western Blot. Furthermore, we try to clarify the role of apoptotic microRNAs, such as miR125b and miR155, in cellular death induced by naringenin derivatives.

Results
Our results indicate that all compounds activate Bcl-2 and Bax expression but ratio Bcl-2/Bax only increases in HT29 cells after treatment with naringenin and 7,4'-di-O-butylnaringenin. In contrast, ratio Bcl-2/Bax decreases following oxime 7,4'-di-O-butylnaringenin treatment and correlates with its strong cytotoxic effect on cancer cells. On the other hand the oxime derivative augments p53 expression at both mRNA and protein level, while naringenin and its O-alkyl derivate do not affect *TP53* gene expression.

Discussion and conclusions
Our study demonstrates the molecular basis for action of novel oxime derivative of naringenin and provides it as a promising agent in anticancer therapy.

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Sonoporation as a novel method for overcoming multi-drug resistance phenomena in human gastrointestinal cancer cells

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Background
Some types of cancers, especially gastrointestinal cancers, display resistance to the chemotherapy which is called multi-drug resistance (MDR) phenomenon. Unfortunately, despite successful surgical treatment, the problem of the cancer cell resistance to chemotherapeutics remains unsolved, resulting in the use of ever-higher doses of cytostatic agents, which often prove to be ineffective. Due to the numerous clinical implications of the MDR phenomenon, there is a crucial need to seek treatments that abolish or modulate MDR. One of them is called sonoporation – the use of acoustic waves for temporary permeabilization of cell membranes, which allows maximization of targeted gene and drug delivery to tumors while minimizing their systemic toxicity. The main aim of this study was to investigate the effect of sonoporation as well as sonoporation with cytostatics, calcium ions, and microbubbles and curcumin in tumor cells of the gastrointestinal tract.

Material and Methods
As a research model we used the cells sensitive and resistant to chemotherapy

from colon adenocarcinoma (LoVo, LoVo Dx) and pancreatic cancer (HPAF-II). Preliminary studies included viability, cell death and cell cycle assays.

Results and conclusions

Preliminary studies have shown that the application of sonoporation in the presence of nanobubbles increases the toxicity of cytostatics. The simultaneous use of sonoporation, bleomycin and nanobubbles allowed for temporary permeabilization of cell membranes and efficient drug penetration into the cells. It has been observed that the effect of the therapy used depends on the physical parameters of ultrasound, i.e. the power and pressure of the acoustic wave. A similar relationship was demonstrated in the case of sonoporation with non-toxic calcium chloride, which caused a significant decrease in cell survival. Based on these findings we claim that sonoporation with appropriately selected parameters and the appropriate concentration of micro- and nanobubbles can increase the therapeutic effect of drugs.

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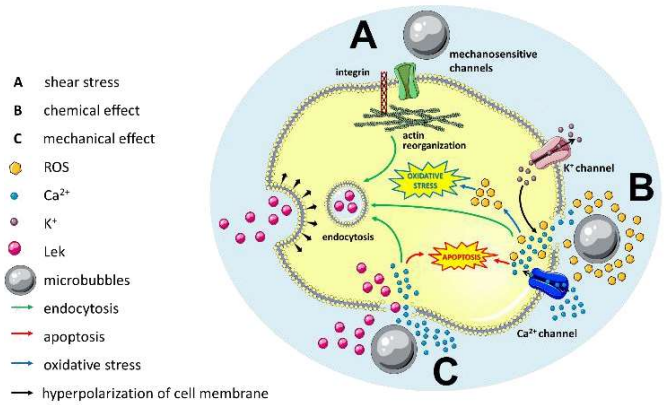

Fig. 1 Cellular effects induced by sonoporation [1]

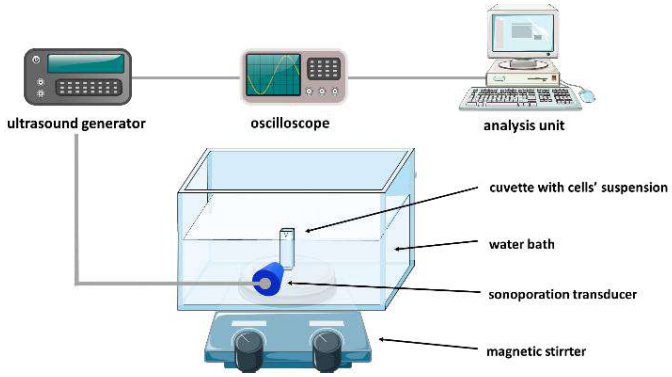

Fig. 2 Setup designed for the sonoporation of cells in suspension

Opposite effects of antioxidants on cancer and normal mammalian cells exposed to subcosmic conditions during the stratospheric balloon campaign

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Background

The current age of dynamic development of the space industry brings the mankind closer to routine manned space flights and space tourism. This progress leads to a demand for intensive astrobiological research aimed at improving strategies of the pharmacological protection of the human cells against extreme conditions. Although routine research in space remains out of our reach, it is worth noticing that the unique severe environment of the Earth's stratosphere has been found to mimic subcosmic conditions, giving rise to the opportunity to use the stratospheric surface as a research model for the astrobiological studies. Our study included launching into the stratosphere a balloon containing mammalian normal and cancer cells treated with various compounds to examine whether these substances can protect the cells against stress caused by rapidly varying temperature, pressure and radiation, especially UV. Due to oxidative stress caused by irradiation and temperature shock, we used natural compounds which display antioxidant properties, namely – catechin isolated from green tea, honokiol derived from magnolia, curcumin from turmeric and cinnamon extract. "After-flight" laboratory tests have shown the most active antioxidants as potential agents which can minimize harmful impact of extreme conditions on human cells.

Material and Methods

Human ovarian cancer cells (SKOV-3; described as "cancer cells") and non-cancer Chinese hamster ovary cells (CHO-K1; described as "normal cells") after 24-hour incubation with various antioxidants were detached, suspended in freezing medium Bambanker™ and placed in microtubes 30 minutes before the balloon flight. Then, the samples were transported on ice to the starting point and placed in a radiation transmitting gondola, located on the environmental measurement unit with accelerometer and temperature, pressure and UV sensors. One half of the samples was covered with aluminum foil to protect the cells against irradiation – mostly UV another half was sent into the stratosphere without the protective layer. As a result, we were able to evaluate the effect of radiation on examined cells in the presence of various antioxidants. As a controls we used not treated with antioxidants and not sent into the stratosphere samples, which were incubated at 37°C, 5% CO^2 during the flight. Directly after landing, biological samples were transported on ice to the laboratory, where after-flight tests were performed (see Fig.1)

Results

The biological samples were launched to the stratosphere on the 30th of April 2018, from Wrocław, Poland (51°06'23.6" N 17°03'32" E). At the highest altitude, the temperature reached the lowest level of -35°C and the lowest pressure (1252 Pa) was measured. Data provided by two UV sensors showed extreme exposure to the UV radiation causing immediate damage of unprotected human skin and eyes. In the upper parts of the atmosphere, the UV dose was more than twofold the dose correlating with the maximum dose in the UV-Index scale (reaching nearly 2463 mV).

Our work has led us to a conclusion that the application of the carefully selected compounds enables us to manipulate cellular stress response depending on the type of cells. Final conclusions about the highest protective potential should be drawn based on the genotoxicity assays and cell death assays. Altogether, these findings suggest that honokiol and catechin have the best protective effect on the normal cells, whereas curcumin and cinnamon act as radio- and light-sensitizers increasing the percentage of apoptotic cancer cells and DNA damage.

Discussion and conclusions

The results constitute a significant step towards the investigation of possible strategies for the cell protection in space environment and provide new insights into the application of the examined compounds for the prevention and treatment of cancer. Due to its relatively low costs, our approach remains the economic alternative for simulated subcosmic conditions conducted in the laboratory, which require far more expensive, specialized measurements.

References:

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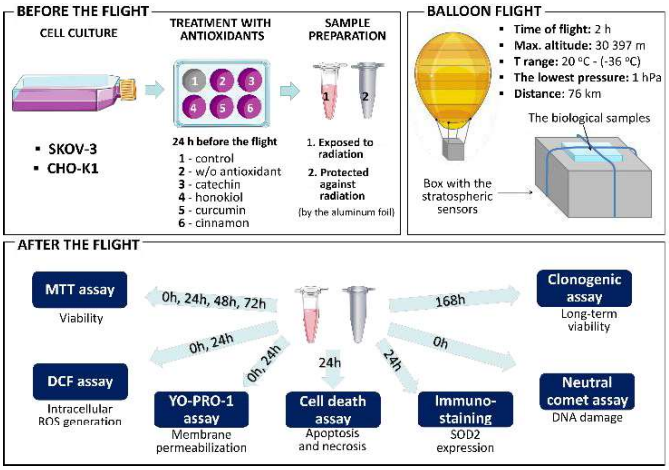

Fig 1 Procedure of experiment and balloon flight

The establishment of insulin resistance model in L6 cell

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Background

Recent studies show that insulin resistance (IR) can precede the development of type 2 diabetes by several years. It is described as the condition in which the sensitivity of peripheral tissues to insulin deteriorates. As a result, the pancreas produce more and more of this hormone, the tissues become more resistant and glycaemic remains unstable. Currently there's no specific insulin-sensitizing drug which could reverse IR symptoms.

Muscle cells can be submitted to any treatment that may have an impact on insulin sensitivity, and glucose uptake measurement will quantify this impact [1]. The aim of this study was to demonstrate induced insulin resistance model.

Material and Methods

The experiment was conducted using rat skeletal muscle cell line L6. L6 myoblasts were induced to differentiation and myotube formation. There was assessed translocation of glucose transporter 4 (GLUT4) to plasma membrane by immunocytochemistry method. The measurement of glucose uptake on cultured myotubes was done to evaluated impact of different conditions and medicines. At the same time, an MTT test was performed to assess the effect of variable conditions on cell survival.

Results

We showed that by stimulating L6 cells with high glucose and high insulin doses we successfully established *in vitro* IR model. There was noticed the translocation of glucose transporter 4 (GLUT4) to plasma membrane.

Discussion and conclusions

The *in vitro* model is suitable for the test of any compounds that could improve insulin responsiveness, or could prevent or reverse acquired or induced insulin resistance [2, 3].

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Evaluation of tumor stem cells in LoVo, HT29 and MCF7 cell lines in three-dimensional cell cultures

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Background

Cancer stem cells (CSC) constitute a small cells population that, due to their pluripotent properties, are able to differentiate into any type of cancer cell. Nowadays, many studies emphasize a key role of CSC in the processes of tumor metastasis, angiogenesis and resistance to cancer therapy, which is important in the formation and evolution of tumors [1, 2].The cells within the spheres can proliferate freely in every direction, similar to *in vivo* conditions. These 3D tumor cells, due to the specific culture conditions, should demonstrate more CSC characteristics comparted to 2D culture. Increased expression of CD44, CD133 and CD326 antigens indicate the native nature of cancer cells growing in spheres.

Material and Methods

We used three-dimensional (3D) cell cultures, due to the possibility of reproducing the most similar conditions of tumor growth by imitating tumor formation. In our work, we develop new 3D cultures so-called spheres based on special hydrophobic powder, which separates the medium containing the cells from the environment. The cells within the spheres can proliferate freely in every

direction, similar to *in vivo* conditions. Afterwards we used flow cytometry to indicate sought antigens (CD44, CD326, CD133).

Results

Here we compare the expression level of antigens: CD44, CD133 and CD326 in 3D cultures of colon and breast cancer cells, sensitive and resistant to the cytotoxic drugs.

Discussion and conclusions

The development of new 3D cultures enriched in CSC population can be helpful in developing new therapeutic strategies for solid tumors. In addition, CSC analysis can contribute to a better understanding of the mechanisms of resistance to chemotherapeutic drugs and to the prevention of the chemoresistance, and thus to the improvement of the effectiveness of currently used oncological therapies.

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Biological Properties of Dental Materials

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Background and aim

Dental materials belong to the medical devices, which have a contact with human body in different time intervals (short period few minutes until few days) or long when they affect the human body for a long period of time – years. The aim of this study was to compare cytotoxic properties of several dental materials on the cell cultures.

Material and Methods

There were verified six different products from SpofaDental portfolio were chosen for the study, details are shown in Tab 1. Samples as a discs with thickens 1 mm and diameter 5 mm were prepared for tests. For example alginate impression materials powder was mixed with water and set at room temperature. Acrylic dental resins were used after mixing of powder (PMMA) with liquid (MMA). Samples were tested for the cytotoxic properties by evaluation using the MTT (methyl thiazolyl diphenyltetrazolium bromide, Sigma) on VERO CCL-81 (ATCC) cell line. Cells were maintained in MEM medium containing 4% FBS in 37°C in humified atmosphere enriched by 5% CO_2. Before exposition cells were suspended on 96-well plate in density $1x10^4$ cells/100 µl in MEM/well. The cells were cultivated for 24 hours to obtained 80% of confluency, and then were exposed to tested samples, positive and negative controls after 24 hours. Cell medium was removed and 6x 100 µl of the sample, positive control, negative control, blank samples were added to individual wells. The test article extract was prepared in 1x MEM cell growth medium (MEM supplemented with 10% fetal bovine serum extract) at the sample to extraction medium ratio of 6.0 cm^2/mL and extracted at 37 ± 1°C for 72 ± 2 hours. The sample was unchanged by the extraction procedure and the extract was found to be clear and free of particulates. After the incubation time with samples, MTT test was applied using in final step 2-isopropanol (100 µg/l/well) with simultaneous shaking. The absorbance was

detected at 570 nm. Based on the acceptance criteria for the procedure, there was judged that the viability of the cells is more than 70% after application of the test sample during 24 hours, what is assumed that material has not cytotoxic properties..

Table 1. Tested materials.

Material	Type of medical device
Elastic Cromo	Class I contact time < 5 min
F.I.T.T	Class I contact time <3-5 days
TAB2000	Class I contact time <30 days
Adhesor	Class IIa contact time long few years
Superacryl Plus	Class IIa contact time 2-5 years
Mifam Super Lux	Class IIa contact time 2-5 years

Results

The obtained results are shown in Fig. 1. The negative control (MEM) and blank sample (MEM with 4% FBS) both demonstrated no cytotoxic effect, thus cell oxido-reductive potential was undisturbed. The positive control (Sodium lauryl sulphate) demonstrated significant cytotoxic impact even after the shortest time of incubation (2h). The viability was less than 20% after 24 hours. Additionally, the cytotoxic effect of positive control has to be proven in all concentrations and negative control has to prove no cytotoxic potential. The most cytotoxic occurred Adhesor sample (c.a. 5%) and the highest biosafety was observed for Mifam, Superacryl, and TAB 2000 (more than 70%).

Conclusions

In dental technology are using different kinds of materials. Some of the has no cytotoxic properties (hot cured acrylic resins). Determination of cytotoxicity is one of the step in the development of new dental materials, required normatively. The results obtained allow the selection of appropriate raw materials. When cytotoxicity is achieved, this does not completely cross out the product being developed as a Medical Device, but requires further appropriate testing. Because in the mouth materials can have a completely different effect than on isolated cell cultures. Such example is material based on ZnO/ phosphoric acid, which have been successfully used for over 150 years in dentistry (the first cement). Often, the solvents (ethanol) contained in the material are responsible for the cytotoxic properties as in the product F.I.T.T.

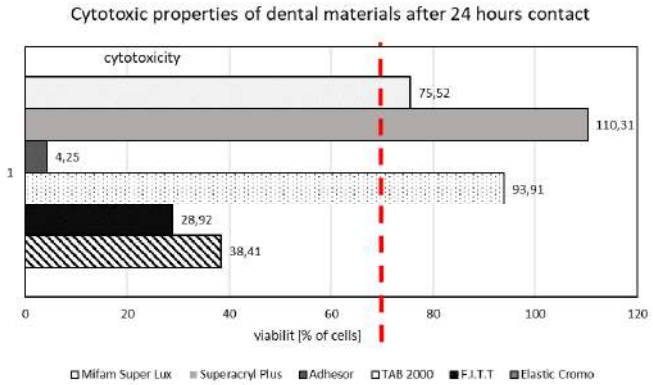

Figure 1. Cytotoxic properties of dental materials are varied and connected with the composition

Atorvastatin aided steroidogenic cells calcium electrochemotherapy

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Background

Plasma membrane associated cholesterol increases its resistance to electric pulse and provides a proper environment for functioning of membrane proteins, some of which like flotillin-2, play an important role in cancer progression and survival [1]. These localise in cholesterol-rich structures called rafts [2]. This crucial role of cholesterol is especially significant among steroidogenic cells [3]. The aim of this project is to validate, if the blockage of crucial for cholesterol biosynthesis enzyme HMG-CoA reductase, can enhance the therapeutic effect of microsecond calcium electrochemotherapy in comparison to control cells.

Material and Methods

MDA-MB231 (breast cancer), Du-145 (prostate cancer) cell lines were used as the model of steroidogenic cells, whereas A375 (melanoma) was a control cell line with high cholesterol content in the membranes, but without the ability of steroid synthesis. To decrease the cholesterol biosynthesis, the cells were incubated for 2 days with atorvastatin. Afterwards, microsecond electrochemotherapy with calcium ions was performed (ESOPE protocol). The response to therapy was compared with the cells, that have not been treated with the inhibitor. The viability of the cells was assessed by MTT assay, the permeability of the membranes was studied with the use of flow cytometry with PI. The adhesive properties of the cells were tested with scratch tests and immunofluorescence studies. Moreover, the model of increasing resistance of the cell membrane with the increasing cholesterol concentration was build with the use of molecular dynamics simulations.

Results

By including calcium ions in the electroporation buffer, the viability of the cells could be significantly decreased in comparison to the standalone electric pulse. Molecular dynamics studies indicate, that by the decrease in cholesterol content, the EP threshold decreases as well. The inhibition of the cholesterol biosynthesis had a high impact on the steroidogenic cells in comparison to the control cell line. The adhesive properties of the cells have also changed in response to atorvastatin-aided calcium electrochemotherapy.

Discussion and conclusions

The decrease of cholesterol biosynthesis could be used as an adjuvant in novel chemotherapy approaches, such as electrochemotherapy. Even though, the preliminary results are promising, further studies need to be done in this field in order to apply this approach in clinical trials.

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This research was financially supported by the Ministry of Health subwention acoording to number of STM.D260.20.142 from the IT Simple system of Wroclaw Medical University.

Lipase-catalyzed synthesis of feruloylated lysophosphatidylcholine

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Background

The pro-health importance of phenolic acids in human nutrition and disease prevention has been well recognized and scientifically confirmed. One of the most abundant phenolic acid, with a wide array of therapeutic properties and lack of side known effects, is ferulic acid (FA) (4-hydroxy-3-methoxycinnamic acid). In the resent years, the number of reports on the *in vitro* anti-inflammatory, antidiabetic, anticarcinogenic, antimicrobial and hepato-, cardio- and neuroprotective [1] properties of ferulic acid has increased significantly. Despite therapeutic effects FA is not use in industry on the high scale. The main reason is his low bioavailability due to intensive transformation to the secondary metabolites in the human body. In studies on the rat model, it has been proven that the orally administered free form of ferulic acid is already absorbed from the stomach in 74% from where is transferred to the liver via the portal vein, there is converted into sulfonic and glucuronide derivatives, which are excreted subsequently via mainly urine [4]. The result of such rapid transformations is low content of free form of ferulic acid in the general circulation. These data were also confirmed in studies carried out on people. Yang and co-workers analyzed the pharmacokinetic parameters of FA following administration of oral FA (50 mg), as a sodium salt (FA-Na). The maximum concentration of free FA in the blood was only 2.5 µmol/L after 24 minutes and its half-life was only 42 minutes [5].

The latest research is therefore focused on improving their physical and chemical properties, which would allow practical application of the ferulic acid in the industry. Promising in this respect are modifications of the FA structure via covalent bonding them with a lipid carrier, especially with phosphatidylcholine, which is characterized by high bioavailability in the human body.

The aim of our work was to develop of a biotechnological method for the preparation of feruloylated phospholipids via a one-step enzymatic reaction. A novel route for the synthesis of PC structured with ferulic acid by enzymatic acidolysis and interestrification of natural egg-yolk phosphatidylcholine was investigated. Enzyme screening, effects of feruloyl donors (FA and EF) and reaction variables (organic solvent, enzyme load, reaction time and substrate ratio) were also evaluated in the process of production of FA-enriched PLs.

Material and Methods

Ferulic acid was subjected to lipophilization with a natural phosphatidylcholine isolated from egg-yolk by enzymatic acidolysis/ interesterification in an organic solvent environment. For this purpose, at the beginning of the experiments, commercially available lipases were tested for their activity to catalyze the regioselective

incorporation of phenolic acid residues into the sn-1 position of natural phosphatedylcholine. Next organic solvent and effects of acyl donors (FA and EF) were evaluated. Then the response surface methodology with 3 factors at 3 levels; substrates molar ratio, enzyme loading and time of reaction, wre used for optimization of reaction of interesterification of PC with EF. Modified phospholipids were purified and analyzed by means of chromatographic methods (SPE, column chromatography, TLC, GC, HPLC). The structure of obtained product was confirmed by spectroscopic data.

Results

We investigated and optimized the process of one-step enzymatic synthesis of structured phosphatidylcholine (PC) ewith ferulic acid (FA). Four different immobilezed lipases were evaluated as biocatalysts for this purpose. Novozym 435 and a binary solvent system of toluene/chloroform 9:1 (v/v) were found to be the most suitable biocatalyst and medium, respectively, which significantly increased the incorporation of FA into PLs fraction. The selected optimized parameters were set as 1/15 molar ratio PC/EF, 30% (w/w) of the enzyme load and 6 days of incubation time. The maximal experimental incorporation of FA into phospholipid fraction (PC/LPC) was 18 mol%. The process of interesterification of egg-yolk PC with EF catalyzed by Novozym 435 at the optimized parameters carried out in the large scale afforded feruloylated lysophosphatidylcholine (FLPC) in high 62% isolated yield. This is the first study reporting the preparation of FLPC by one-step enzymatic method, which is a promising in the context of the production of food supplements and additives based on the phenolic acids and natural PC.

Discussion and conclusions

A novel biotechnological route of incorporation of ferulic acid (FA) into phospholipids was successfully developed. Presented method is then promising in the area of enzymatic production of phospholipid biopreparation containing biologically active ferulic acid (18% of incorporation) with potential application in the food industry as food ingredients, natural emulsifiers or nutraceuticals with proved pro-health activity.

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Expression of neuronal isoform of nitric oxide synthase (nNOS) in the porcine enteric neurons of jejunum following prolonged indomethacin supplementation

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Background

The enteric nervous system (ENS), also called the second brain, consists of a mesh-like system of neurons and is capable of acting independently of the sympathetic and parasympathetic nervous systems. The ENS, outside the central nervous system, is one of the main regulators of gastrointestinal functioning and contributes to tissue response to the various pathological conditions [1].

Indomethacin is a nonsteroidal anti-inflammatory drug (NSAID) commonly used to reduce fever, pain, stiffness, and swelling from inflammation.

The goal of the present study was to determine the influence of high doses of indomethacin on the neurons in the enteric nervous system of the porcine jejunum. It should be noticed that selection of the drug active substance and animal species model were not accidental. Swine is considered to be one of the main type of animals in biomedical researches.

Material and Methods

The experiment was performed on 8 immature gilts of the Pietrain x Duroc breed weighting approximately 20 kg and around 8 weeks old. The animals were divided into 2 groups – control (n=4) and experimental (n=4). The gilts constituting control group received empty gelatin capsules orally, while pigs from the study group were given indomethacin orally 10 mg/kg of body weight. The experiment lasted 4 weeks, then pigs were euthanized. After fixation and freezing section, double immunofluorescence staining was performed. Antibodies against the protein gene-product 9.5 (PGP 9.5) neuronal marker and against the nNOS were used as primary antibodies, while Alexa Fluor 488 and 546 were used as secondary antibodies. Stained 14 μm sections were examined under Olympus BX51 fluorescence microscope.

Results

In the present study supplementation with indomethacin caused changes in the neurochemical phenotype of nerve cells. Analysis of the obtained results showed that inflammation caused by long-term administration of high doses of indomethacin resulted in decrease of the number of nNOS positive neurons in the myenteric and submucosal ganglia in the porcine jejunum.

Discussion and conclusions

The present immunohistochemical studies revealed that in the jejunum of pigs treated with high doses of indomethacin subpopulations of nNOS-immunoreactive myenteric and submucous neurons were statistically altered. Previous studies have also shown similar changes in this neuroactive substance in the swine gastrointestinal tract during induced inflammatory processes [2, 3]. It is believed, that the capacity to change neurotransmitter/neuropeptide content, termed enteric neuroplasticity, is an adaptation to an unfavorable enteric microenvironment [4].

Changes of nNOS expression in enteric neurons suggest the neurochemical adaptation of these neurons to the conditions of induced inflammation and possible involvement in local repair processes.

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Osteoprotegerin in carotid atherosclerosis – preliminary research

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Background

Carotid artery stenosis is a main reason of stroke and cognitive dysfunction. Plaque instability is responsible for acute cardiovascular events. One of the elements of plaque vulnerability is calcification, which is regulated *via* osteoprotegerin (OPG)/receptor activator of NF-κB ligand (RANKL)/receptor activator of NF-κB (RANK) system and OPG is a candidate biomarker of atherosclerosis.

The aim of the study was to assess the plasma concentration of OPG in patients with carotid stenosis and its correlation with routine laboratory test results. We also aimed to evaluate the OPG tissue content in parts of artery with atherosclerosis.

Material and Methods

20 patients after routine carotid endarterectomy at the Department of Vascular Surgery of 4th Military Teaching Hospital in Wroclaw were enrolled in this study. The OPG concentration was measured in:

- part of removed artery with visible calcified and ulcerative changes – "plaque",
- part of removed artery without visible symptoms of atherosclerotic lesions – "reference tissue",
- citrated plasma collected before surgery.

The OPG concentration was determined by ELISA. Prior to analysis tissue fragments were homogenized in liquid nitrogen and then lysed with homogenization buffer and Pellet Pestle® Motor. The laboratory results were routinely performed before surgery in a hospital laboratory. The statistical analysis was performed in GraphPad Prism 5.

Results

We observed higher amount of osteoprotegerin in reference tissue than in the area of the plaque ($p = 0.0009$) (Figure 1).

There was no correlation between plasma OPG and laboratory test results as presented in Table 1.

Table 1. Correlation between routine test results and OPG in plasma.

Parameter	Correlation	Parameter	Correlation
Total cholesterol	r = 0,2791 p = 0,2335	Triglycerides	r = 0,09936 p = 0,6768
LDL-cholesterol	r = 0,225 p = 0,3403	White blood count	r = -0,3164 p = 0,1741
HDL-cholesterol	r = 0,1734 p = 0,4647	Platelet count	r = 0,3544 p = 0,1252
Non-HDL-cholesterol	r = 0,2143 p = 0,3644	Creatinine	r = -0,1964 p = 0,4066

Discussion and conclusions

Strong calcification in the core of the plaque may be a result of smaller amount of OPG, because OPG is an inhibitor of vascular calcification [1,2]. Hakimi et al. showed that OPG was the highest in the marginal part of the plaque (assessed by immunohistochemical staining) [3]. We suppose that lower amount of OPG in the middle part of the lesion may be also a result of OPG degradation by proteases released from inflammatory cells massively infiltrating the plaque. OPG has been extensively investigated and its diagnostic potential as a marker of aortic plaque presence or plaque vulnerability is well documented, however, osteoprotegerin has not been included in routine use so far and further research is required [2, 4].

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List your references here, and use the example below:

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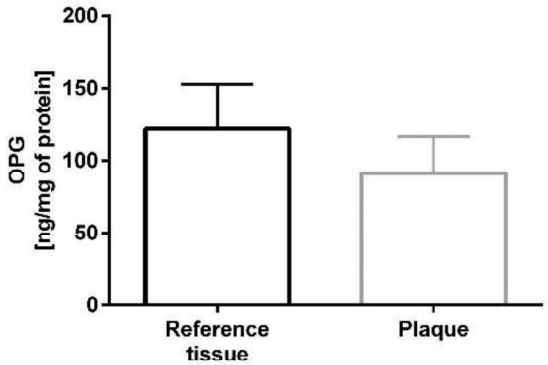

Figure 1. Comparison of the content of OPG in tested tissue fragments

Determination of methanol, ethanol and isopropanol in the gas phase using IR spectroscopy

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Background

The The aim of this work was to develop an analytical method to detect methanol, ethanol and isopropanol in the gas phase using IR spectroscopy. The procedure involves recording the Fourier-transform infrared spectroscopy (FTIR) spectra of alcohols in gas phases and also determination of analytical wave numbers and plotting the dependence of the absorbance on the pressure of the analysed component. The reliability of measurements was checked in the presence of other gaseous components, i.e. nitrogen, air.

Material and Methods
Measurements were carried out in a cell filled with a gaseous form of the tested alcohols in a vacuum apparatus under controlled pressure to $1 \cdot 10^{-5}$ atm.

Results
The results of the research allowed to determine several analytical wave numbers useful in quantitative measurements of the tested compounds in the pressure range from $6{,}5 \cdot 10^{-4}$ atm. to $1{,}3 \cdot 10^{-1}$ atm. ($2{,}7 \cdot 10^{-5}$ mol/dm^3 to $5{,}4 \cdot 10^{-3}$ mol/dm^3). The calibration curves were characterized by high linearity. It was found that quantitative measurements of alcohols are also possible in the presence of other gases, e.g. nitrogen, air and in a mixture of alcohols.

Discussion and conclusions
Alcohols used in the work are the most commonly used solvents for the extraction of plant materials. Although FTIR is a technique that is rarely used for the quantification of residual solvents [1], it can be proposed for rapid initial evaluation of the residues of some solvents used in pharmaceutical procedures.

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Psychophysiology of empathy – galvanic skin reaction

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Background
The term "empathy" was first used by Titchener in 1909 [1] and has been present in colloquial language, literature and art ever since. Despite deep cultural rootedness, both a widely recognized definition and effective explanation of its biological foundations are lacking [2]. The first person to conduct the research on empathy in the context of psychophysiology, using galvanic skin reaction as the main measurement, was probably Dennis Krebbs, who published his results in 1975 [3]. Although the author managed to combine the strength of the momentary empathic response with an increase in GSR readings, the methodology he used is controversial, because he completely omitted empathy-trait as a variable.

Several modern studies confirm the existence of a relationship between the skin galvanic response to emotive stimuli and the level of empathy [4, while others reject this thesis [5]. The inconsistency of the results of previous studies leaves the question about the possibility of linking GSR with empathy open. Answering it would open up new possibilities in objectifying the measurement of empathy.

Material and Methods
The measurement of empathy was conducted using the Polish adaptation of Baron-Cohens' EQ-short scale (SSIE) [6] and the Empathic Sensitivity Scale (SWE) [7].

Galvanic skin reaction was measured using a galvanometer included in the Stoelting polygraph.

The empathic stimuli used in the research were taken from Affective Picture List [8] and Nencki Affective Word List [9] and additionally checked in a pre-test.

A total of 65 students were examined in the study.

The study received a positive opinion of the Scientific Research Ethics Committee of the

Institute of Applied Psychology, Jagiellonian University.

Results

GSR levels were found to be the highest when subjects were presented with negative emotive stimuli ($p<0.001$) and lowest when the stimuli was positive ($p=0.011$). A total of 15 analyses were conducted to check the relationship between each of the scales of the two empathy questionnaires and galvanic-skin reaction to each type of the stimuli.

As a result of multiple regression analysis, it was found that the general SWE result is a predictor of the strength of skin-galvanic reaction strength to negative stimuli ($r = 0.54$; $p = 0.02$), and the general SSIE result is a negative predictor of skin-galvanic reaction strength to positive stimuli ($r = -0.41$; $p<0.01$). The remaining 13 relationships were found to be statistically insignificant ($p> 0.05$).

Discussion and conclusions

Although it was proven that different types of emotive stimuli evoke different levels of galvanic-skin reaction, the authors were unable to link the strength of the GSR to empathy levels.

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Influence of fluoride on endocrine tissue

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Environment pollution is a serious problem for developed and developing countries. Anthropogenic pollution results in the constant emission of hazardous substances that contaminate water, air and soil, leading to such problems as global warming and smog, but also – the increase of the concentration of heavy metals and toxic elements, including fluorine. Dutkiewicz placed fluorine on the list of top 5 of the most dangerous environmental toxins as early as in 1995. Therefore, it is important to investigate and trace substances which contribute to the development of disorders in the human body

The first observations suggest that fluoride has a negative influence on the functioning of the thyroid, ovaries and testicles. The current state of knowledge suggests a significant effect of this element on the decrease in sex hormone levels, which may in turn cause impairment of fertility and puberty. Most studies confirm that sodium fluoride causes an increase in TSH concentration and a decrease in the concentration of T3 and T4 secreted by the thyroid glands. In addition, there were correlations between NaF and an increase in parathyroid hormone secretion without significant effects on body calcium. It is possible that fluoride adversely affects the amount of insulin leading to impaired pancreatic function resulting in impaired glucose tolerance. A decrease in cortisol secreted by the adrenal glands was also observed.

There are observations that indicate fluoride its toxic influence on the endocrine system, but so far this phenomenon has not been documented in detail. The correlation between potential inflammation within these organs and the amount of hormones released to the bloodstream seems to be particularly interesting. In recent years, you can see an increase in the incidence of autoimmune diseases, including Hashimoto's disease. Despite limited research, no undeniable pathway for endocrine toxicity has been found, that's why we should look for the reasons for these upward trends.

Nanoemulsions in the treatment of the vaginal infections

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Background

Infections of the female reproductive system are still a challenge for modern medicine. Oral drug administration forces the use of increased doses. It has a negative influence on patients' health, increases the risk of complications and superinfections caused by a different microbial agent. Intravaginal route of drug administration provides higher bioavailability due to rich vascularization and avoiding first past effect through the liver and intestine. However, individually and temporally changing the composition of vaginal mucus are the limiting factors of the possibility of intravaginal drug administration.

Nanoemulsions are a thermodynamically unstable colloidal dispersion commonly consisting of oil in water droplets sized <300 nm. They are preferable over thermodynamically stable microemulsions due to their kinetic stability which is less affected by the change of conditions e.g. during the administration of the drug to the patient [1,2]. Nanoemulsions present a high degree of adhesion to the mucosa and simultaneously allow delivery of several active pharmaceutical ingredients (APIs) of different hydrophilic or lipophilic properties,

which makes them potential drug form for intravaginal administration.

The aim of this study is determining the current state of knowledge in the intravaginal application of nanoemulsions in the treatment of female reproductive tract infections.

Material and Methods

Relevant papers were searched in the Web of Science and Scopus databases. The scope of the research has been limited to records published in years 2014-2019. Different forms of words: vaginal, intravaginal, emulsion, nanoemulsion were used as the keywords.

Results

Among the reviewed papers, two main types of formulations can be distinguished – the nanoemulsions and emulgels based on nanoemulsions and two groups of constituent activity – antifungal and antibacterial.

Natural lipid fractions such as cholesterol, oleic acid, and soybean oil were used as an oil phase, as well as synthetic like Labrafac® Lipophile.

As an API in the treatment in vaginal candidiasis oxiconazole nitrate, clotrimazole, *Syngonanthus nitens* extract and *Mentha spicata L. var. viridis* aromatic oil was used. Polyphenon 60, ciprofloxacin, cranberry and curcumin were selected as an antibacterial agent.

In addition to zeta potential, droplet sized of every obtained nanoemulsion were characterized by dynamic light scattering methods. Particle size varied from 23 nm to approx. 300 nm. Most of the researchers provided SEM or TEM images and rheological measurements were performed. *In vitro* drug release tests were varied between groups – Franz diffusion cells, dialysis bags and USP dissolution test apparatus II were used. In some cases, construction of ternary phase diagrams was used in the technological part of the research. Only in a few articles information about the pH range of the formulation was provided ranging approx. from 3 to 6 [3-9].

Discussion and conclusions

Nanoemulsions enhance the effect of simultaneous administration of hydrophilic and lipophilic APIs. Based on *in vitro* and *in vivo* studies, natural-origin and synthetic antimicrobial compounds showed promising results in the treatment of both bacterial and fungal vaginal infections. Most of the formulations present modified release profile. Yet, further studies are needed.

It would be favourable to prepare analytical guidelines for the research on intravaginal nanoemulsion as there are not many requirements in European Pharmacopeia for these formulations. There are significant differences the between analytical methods used to determine the properties of intravaginal nanoemulsions.

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Biogenic and pyrogenic SiO_2 nanoparticles – which of them are safer for endothelial cells?

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Background

Silica is a key component of almost every species of plants and animals. Silicon dioxide in the form of nanoparticles posssesses some unique physico-chemical properties which make these nanoparticles a platform useful in biology and medicine. Moreover, due to their size, shape and large surface to volume ratio, these nanoparticles are the most promising material used as an adsorbent, filler, and additive to drugs and cosmetics [1]. Furthermore, due to their biocompatibility, the silca nanoparticles can also be use as biosensors [2], biomarkers and drug carriers [3]. In this paper, we compared the biological effects of both silica nanoparticles extracted from *Urtica dioica L*. and pyrogenic, *i.e* commercially available silica nanoparticles.

Material and Methods

Biogenic and pyrogenic silica nanoparticles were studied using SEM and TEM. The studies were conducted immortalized human microvascular endothelial cells (HMEC-1). The cells were cultured in MCDB 131 medium under 5% CO_2 in plastic flasks at 37ºC.

The cytotoxic effects of NPs on cells were determined after exposure to different concentrations (0-200 µg/ml) at 24, 48 and 72 h. The cell viability was measured using fluorimetric (Hoechst 33258) assay. The ability of the compounds cell cycle arrest was studied using flow cytometry analysis. The cells were stained using FxCycle PI/RNase Staining Solution.

Results

The study, which was conducted using transmission electron microscopy (TEM) and scanning electron microscopy (SEM), confirmed that the size of tested silica is between 8 and 20 nanometers as well as its amorphous structure. In terms of chemical composition, we report that the nanoparticles obtained by „green chemistry" method (*bio*SiO_2) have similar composition to synthetically produced (*pyr*SiO_2).

The obtained data indicated that SiO_2 NPs extracted from stinging nettle show higher toxicity than pyrogenic NPs in immortalized human microvascular endothelial cells (HMEC-1). Furthermore, the level of cytotoxicity is time and concentration dependent. In the current study, we confirmed that the exposition on *pyr*SiO_2 did not elicite statistically significant cell cycle arrest at the G2/M cell cycle phase.

Another effect was observed after treatment of cells with *bio*SiO_2 nanoparticles. Collected data indicated on decreasing level of cells in the G2/M cell cycle phase. This may suggest that nanoparticles caused excessive oxidative stress (which was confirmed in previous study) and they are toxic for HMEC-1 cells.

Discussion and conclusions

In terms of chemical composition, we found that the particles obtained from an extracted form of *Urtica dioica L*. have similar composition to synthetically produced silica. Nanoparticles of biogenic silica obtained from plant material are considered to be a potential source of nanomaterial usable for many applications. Silica nanoparticles can cause oxidative stress, leading to DNA damage, cell cycle arrest, and apoptosis [4,5]. What is more, toxicity of silica nanoparticles depends on type of nanoparticles, their size, dose, and cell type [6]. In addition, the results proved that both biogenic and synthetically produced nanopartciles are safe for endothelial cells in appropriate concentration (50 µg/ml) and show good biocompatibility which makes them promising candidates for future studies.

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Acknowledgements

This work was supported by grant for the development of young scientists no. B030/0032/19 and from funds of the statutory activities of the Department of Physics and Biophysics, Wroclaw University of Environmental and Life Sciences, grants no. B010/0041/19.

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Variability of serum immunoglobulin G degree of galactosylation in women with endometriosis – pilot study

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Background

Endometriosis is an inflammatory disease characterized by the presence of endometrial tissue, outside of the uterine cavity, changed in its architecture, and has been associated with a wide range of factors. Diagnosis of endometriosis is solely made through surgery/laparoscopy as does not exist consistent biomarkers for disease diagnostics [1]. Immunoglobulin G (IgG) is a main human serum protein, glycoprotein which is a powerful effector molecule that can mediate tissue inflammation by complement activation. The agalactosylated form of IgG act as proinflammatory factor, and the increased expression of agalactosylated forms of IgG is observed in some inflammatory diseases, e.g. in rheumatoid arthritis [2].

The aim of our study was the analysis of serum IgG degree of galactosylation/agalactosylation in women suffering from endometriosis.

Material and Methods

The serum samples – study group – were collected at the Department of Gynaecological Oncology in Lower Silesian Cancer Centre (Poland). A healthy control group consisted of serum samples from women without endometriosis. The samples were divided into two groups: the control group (n=10) and the group with endometriosis (n=28). The degree of IgG galactosylation was determined using a modified solid phase enzyme-linked immunosorbent assay, lectin-ELISA. The method is based on the relative reactivity of IgG glycans with specific biotinylated lectins: *Ricinus communis agglutinin I* (RCAI) – detect the terminal galactose; *Griffonia simplicifolia II* (GSL-II) – detect the terminal GlcNAc. To assess the galactosylation status of IgG N-glycan's, the agalactosylation factor (GSL-II/RCA-I) was calculated [2]. Statistical analysis was performed using STATISTICA 13.3 PL (StatSoft Inc.) software (U Mann-Whitney test).

Results

The results of statistical analysis show significant differences in the relative reactivity of IgG oligosaccharides with lectins used, between the control group (healthy women) and the group of women suffering from endometriosis ($p < 0.005$). The agalactosylation factor was significantly higher in group of patients with endometriosis, than in group of healthy women ($p < 0.005$).

Discussion and conclusions

Due to the fact that IgG effector functions are controlled by N-glycosylation, its altered galactosylation and agalactosylation status can contribute to immune dysregulation in chronic inflammatory diseases such as endometriosis [3]. The ratio of GSL-II/RCA-I relative reactivity, calculated for serum IgG glycans, may become an additional diagnostic marker of endometriosis.

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Quantum chemistry aided examination of antioxidative potential of phenolic acids

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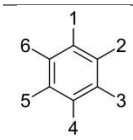
Background

Phenolic acids belong to the group of polyphenolic compounds known for their health-promoting effects resulting from the antiradical activity [1].

The very main determinant of polyphenols' scavenging potential is a presence of hydroxyl groups linked with the aromatic ring, their substitution pattern and additional residues [2].

In this study antiradical activity of phenolic acids was investigated and new activity index was proposed. Experimental results and computational quantum chemistry data were statistically corelated.

Table 1 Structures of investigated phenolic acids



Phenolic acid	1	2	3	4	5	6
gentisic	COOH	OH	H	H	OH	H
homogentisic	CH_2COOH	OH	H	H	OH	H
homoprotocatechuic	CH_2COOH	H	OH	OH	H	H
p-coumaric	CH=CHCOOH	H	H	OH	H	H
4-hydroxyphenylacetic	CH_2COOH	H	H	OH	H	H
β-resorcylic	COOH	OH	H	OH	H	H
ferulic	CH=CHCOOH	H	OCH_3	OH	H	H
homovanillic	CH_2COOH	H	OCH_3	OH	H	H
caffeic	CH=CHCOOH	H	OH	OH	H	H
syringic	COOH	H	OCH_3	OH	OCH_3	H
4-hydorxybenzoic	COOH	H	H	OH	H	H
gallic	COOH	H	OH	OH	OH	H
m-coumaric	CH=CHCOOH	H	OH	H	H	H
protocatechuic	COOH	H	OH	OH	H	H
vanillic	COOH	H	OCH_3	OH	H	H
α-resorcylic	COOH	H	OH	H	OH	H
3-hydroxybenzoic	COOH	H	OH	H	H	H
o-coumaric	CH=CHCOOH	OH	H	H	H	H
2,3-dihydroxybenzoic	COOH	OH	OH	H	H	H
salicylic	COOH	OH	H	H	H	H
sinapic	CH=CHCOOH	H	OCH_3	OH	OCH_3	H
veratric	COOH	H	OCH_3	OCH_3	H	H

Material and Methods

Investigated phenolic acids (see Table 1) were tested with FRAP, ABTS and DPPH methods.

Quantum chemistry pathway consisted of obtaining isomers with the simulated annealing and geometry optimization linked with frequency computations, up to UB3LYP/6-31G(d,p) DFT method [3]. Elaboration of the hydrogen abstraction was performed by successive removal of a hydrogen atom from each hydroxyl or methoxy group and repeating DFT calculations. In order to obtain the reorganisation energy (*RE*) energies of unrelaxed radicals were noted. The polarizable continuum model (*PCM*) of the solvents was applied as well [4].

The hydrogen abstraction energy (*HAE*), describing energy required to remove hydrogen atom from the given hydroxyl group can be ascribed to the equation:

$$\boldsymbol{HAE(p,r) = (min_{\{r,c\}} E_{relaxed}(p,rc) - min_{\{rc\}} E(r,c)) + E(H)}$$

Lai et al. [5] transition state energy, determining the hydrogen abstraction rate, is stated to be:

$$\Delta E = 0.3(RE_r + RE_a) + 0.55HAE - BDE_{aH}$$

To check whether radical reorganisation energy (RE_r) has an influence on hydrogen abstraction kinetics we introduced *HAIndex*. Its main goal is to describe the transition state energy up to some constant, dependent only on the hydrogen acceptor molecule. Thus, the differences between *HAIndex* values should correspond only to the differences in transition state energies of

phenolic acids reacting with the same abstractor in the same reaction medium. *HAIndex* can be described as:

$$HAIndex = 0.3RE_r + 0.55HAE$$

Results

The strongest activity reducing ion Fe^{3+} to Fe^{2+} was noted for 2,3-dihydroxybenzoic acid. A slightly lower activity was observed for homoprotocatechuic acid and gentisic acid. Similar results were obtained for ABTS and DPPH tests. The model of hydroxylation by two hydroxyl group situated next to a carboxyl group seems to be the most effective, alike observation can be made for mutual ortho position, model of 2,5-hydroxylation and hydroxyl groups immediate vicinity to carboxyl residue.

Discussion and conclusions

The results of quantum studies are well correlated to the experimental data – Spearman's rank order correlation coefficient shows strongly negative correlation with a two-tailed p-values less than 0.01 in most cases.

In conclusion, it can be stated that:

Compounds with only methoxy or one hydroxyl group exhibit very low antioxidant activity;

Position C4 seems to be very important for the antioxidative activity of phenols;

Partial methoxylation of cinnamic and benzoic acids at C3 and C5 positions and hydroxylation at C4 promote antioxidant activity.

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Influence of Structural Features on the Antiradical Activity of Flavones and Flavonols — A Quantum Chemical Study

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Background

Flavones and flavonols are representatives of a widespread group of dietary polyphenols, which beneficial activity results from the radical scavenging potential [1]. Presented studies were conducted in order to examine the influence of the structural features on these natural antioxidants' hydrogen donating ability, described here by the enthalpy values of the first step of the proposed mechanisms of action.

Material and Methods

The low-energy geometries of 13 investigated compounds were generated using molecular dynamics simulations. Electronic structure studies were performed starting with the preoptimization at HF/3-21G(d) level of theory, up to B3LYP/6-31+G(d,p)

in a water solvent, using Polarizable Continuum Model [2, 3].

Results

Computational data provided valuable information about the electronic structure of the investigated compounds. Each aspect was deeply investigated and discussed as follows.

Discussion and conclusions

Natural Bond Orbital analysis demonstrated significant influence of p_y orbitals conjugation on the system's energy reduction, manifested in the mutual planarity of rings B and C. Moreover, the contribution of the C2=C3 double bond and carbonyl residue in an electron flow among investigated flavornoids' backbone has been elucidated. Thermochemical calculations indicated that the most prominent mechanism of hydrogen abstraction is Sequential Proton Loss Electron Transfer from C7 hydroxyl group. Consideration of intramolecular hydrogen bonds is remarkably important while describing the formation of the radical, understood as a hydrogen-donating ability – their presence could lower enthalpy up to 7 kcal/mol. Sroka et al. in their experimental studies have shown over a hundredfold difference between the measured activity of apigenin and luteolin [4]. Theoretical studies somehow clarified this unusual behavior with an existence of catechol group in luteolin structure which undergoes two reactions, forming stable o-hydroquinone structure in the end. Appliance of Frontier Molecular Orbital Theory and visualization of the obtained results allowed to refer the electron density distribution to the hyperconjugation effect and the chemical hardness. The mechanism of intramolecular hydrogen swap between C5 hydroxyl group and the carbonyl oxygen of the radical was proposed and investigated – though it could extend delocalization, the studies didn't prove it is likely to occur.

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Spectrophotometric analysis of creatinine in artificial and mixed urine with increased sarcosine content

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Background

Creatinine (CREA) is produced in muscles by irreversible non-enzymatic dehydration and spontaneous cyclization from creatine and (after phosphate cleavage) from creatine phosphate. Creatine phosphate serves as a source of energy in muscle. CREA cannot be re-phosphorylated and passes into the blood and is excreted in the urine. In the body, CREA is formed at a relatively constant rate. Its formation is related to muscle mass and is stable under normal calm and

meatless diet (with normal glomerular filtration). It is very often monitored to assess renal function, but CREA levels have also been monitored in relation to various cancers (ovaria cancer, head and neck cancer, hepatocellular, breast cancer etc [1-4]). For the determination of CREA, the Jaffe reaction is used to react with picrate in an alkaline medium to form a red-orange complex. In this method, other components of biological fluids (pyruvate, acetate, and others) react with picrate. New tumor markers are intensively searched for rapid diagnosis. One potential tumor marker of prostate cancer could be the amino acid sarcosine (SAR). Increased concentrations of SAR were found in the urine of patients with prostate cancer. The aim of this study was to detect CREA in various urine types in the presence of SAR.

Material and Methods

Mindray BS300 analyzer was used for the analyzes. The chemicals used for the analysis were purchased from Merck. Artificial urine was prepared according to published protocols. Mixed urine was prepared by mixing urine of the volunteers (n = 5). Distilled water was prepared by the Aqual system and the ultrapure water was prepared by the ELGA system up to 18 MΩ water quality. The determination of pH was performed on a pH meter. Concentrations of ions were determined by ISE (K, Na, Cl). SAR was analyzed by Trinder reaction on a plate photometer for 30 min. All analyzed data were transferred to the QUINSLAB laboratory database and statistically evaluated.

Results and Discussion

Analysis of CREA is a routine and long-term method in clinical biochemistry. However, slight variations in determination of CREA concentration are not an obstacle to the interpretation of the results. The ability to monitor CREA and possibly another analyte present in the urine may require the determination of the CREA concentration as accurately as possible. There were three main blocks of analyzes in our work: A) dependence of absorbance on CREA concentration in different matrices; B) dependence of absorbance on CREA concentration in different matrices in the presence of SAR (25 µmol/L); C) changes in analytical SAR response (25 µmol/L) in different matrices and CREA concentrations (0-100 mmol/L). Thus, n = 60 replicates of individual CREA concentrations were analyzed. The mean linear dependence was described by the equation: y = 0.091x + 0.0405 (r = 0.9960), QC 12.76 %, RSD 13.83 %. Within the range of lower creatinine concentrations (0-10 mmol/L) the dependence was linear (r = 0.9955), RSD 12.62 %, QC 12.41 %, LOD (0.83) and LOQ (2.75) mmol/L. We found that CREA detection is similar in all studied matrices. In the presence of SAR, no effect on the CREA signal was also observed, and correlation analysis was -SAR, + SAR with r = 0.999. When monitoring the SAR signal as a function of increasing concentration (0-100 mmol/L) of CREA, slight changes in trend (r = 0.2-0.3) were observed in AU-N (artificial urine); trend changes (r = 0.3-0.4) were also observed for AU-7 (artificial urine), and the downward trend in mixed urine 11 was most pronounced (r = 0.55-0.58). When evaluating the data obtained in the control diagram, 90 % of the values were in the 1SD band.

Conclusions

Detection of CREA by the proposed procedure was reproducible in all types of samples analyzed. No changes in CREA detection were observed in the presence of SAR. CREA (30 mmol/L) interfered slightly with SAR and a decrease in signal was observed.

Acknowledgements
The work was carried out with the support of Liga proti rakovine Praha 273/2019.

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Anti-inflammatory and skin regenerating properties of vegetable oils

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Skin is the largest organ of the body and is a barrier between external and internal-environment. Every day, skin is exposed to many harmful factors, such as mechanical damage, pathogens, ultraviolet radiation (UV), allergens and irritants. Skin damage and lack of proper skin care result in inflammatory processes, which can lead to the development of chronic inflammatory skin diseases, e.g. atopic dermatitis.

Plant oils are natural fats extracted from different parts of plants (seeds, fruits or sprouts). Oils, depending on the place of origin and chemical composition, have different beneficial effects on the condition of the epidermal barrier reducing TELW (transepidermal water loss), creating occlusive layers, restoring structures of *stratum corneum* and participating in biological processes, such as hormone synthesis, cell division, inflammatory processes and metabolism of lipids and amino acids.

The aim of this paper is to present a few selected vegetable oils (coconut oil, pomegranate seed oil, rose hip oil, *Calophyllum inophyllum* oil, argan oil) that may be useful in the production of cosmetic and pharmaceutical emulsion with anti-inflammatory and skin regenerating properties.

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Contamination of the API with nitrosamines

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Drug manufacturers have been obligated by the European Medicines Agency (EMA) to carry out a risk analysis of nitrosamines contamination in produced medicines. Over the past two years, a significant amount of nitrosamines has been detected in specific batches of drugs such as valsartan, ranitidine or recently metformin.

It has been shown that the contamination source of valsartan preparations, a popular antihypertensive drug, could be starting materials, reagents and solvents, used in the synthesis of the API (Active Pharmaceutical Ingredient). Valsartan contains a tetrazole ring. The synthesis of this ring requires the use of sodium nitrite, which reacts with amine or trace amine solvents to form nitrosamines. The risk of cancer has been investigated with patients taking contaminated valsartan preparations.

The latest information shows that N-nitrosodimethylamine (NDMA) impurities have also been found in metformin preparations, which may be the result of a technological process. Metformin remains the first-line medication, controlling high blood sugar in patients with type II diabetes, and its abrupt withdrawal may result in health consequences.

Interesting results have been shown in studies on preparations of ranitidine – an H2 receptor antagonist, which is one of the most commonly used pharmaceuticals in the treatment of gastroesophageal reflux disease and peptic ulcers. Urinary NDMA excretion after clinically used doses of ranitidine was evaluated. Researchers confirmed the production of N-nitrosodimethylamine (NDMA), a potent carcinogen, by nitrosating ranitidine at gastric pH conditions. The work is a review of the latest publications on this important topic. Observations made by scientists suggest the need for risk assessment related to NDMA toxicity, the concentration of which can increase significantly in the body with chronic use of these drugs. Therefore, it is extremely important to use alternative treatment methods that minimize exposure to N-nitrosamines.

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Petunidin-3-O-glucoside as a natural antioxidant which is in interaction with the model membrane, human albumin and plasmid DNA

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Background

Anthocyanins are natural dyes which are present in plants as derivatives of β-*O*-glycoside with the most popular substituent *i.e.* glucose and main aglycones including cyanidin, delphinidin, petunidin, malvidin, pelargonidin and peonidin [1]. Conducted studies indicate on great importance of anthocyanins as pro–health food ingre-

dients. Such an opinion is based on many biological activities which are shown by these compounds, in particular antioxidant, anti-inflammatory, anticancer, cardioprotective activities as well as their role in vision improvement, protection against diabetics and against degeneration of neurons [2, 3].

The aim of this research was to investigate antioxidant properties of petunidin-3-*O*-glucoside (Pt-3-glu) as well as to determine the consequences of its interaction with biomolecules *i.e.* mimic lipid membrane, which reflects the lipid membrane composition of tumor cells, human serum albumin and plasmid DNA.

Material and Methods

Antioxidant activity Pt-3-glu was investigated using fluorometric method in reference to mimic lipid membrane in which free radicals were induced in a way of thermal decomposition of AAPH. Using fluorescence probes DPH and MC540, which were located at different depths of a lipid bilayer, the impact of Pt-3-glu on the properties of both hydrophobic and hydrophilic areas within mimic membranes was determined. Determination of binding between Pt-3-glu and albumin was carried out in a way of fluorescence quenching of albumin and using steady state fluorescence spectroscopy. Interaction of plasmid DNA molecules with Pt-3-glu was performed using fluorescence correlation spectroscopy with the function of single photon counting (TCSPC-FCS), enabling tracking of dynamics changes at the level of single molecules, while tracking changes in fluorescence lifetime.

Results

The conducted studies indicated that Pt-3-glu shows high antioxidant activity (IC_{50}=2.44±0.24 μM) as well as it protects lipids against the peroxidation process more effectively than ascorbic acid (IC_{50}=129.44±12.43 μM). Studies based on the use of fluorescent probes showed that Pt-3-glu is mainly localized on hydrophilic part of bilayer and is responsible for increasing rigidification of this part of a membrane. Such a location of Pt-3-glu in hydrophilic part of a membrane may be a sort of a barrier which protects a membrane against the detrimental impact of free radicals. In addition, stiffening effect of lipid molecules may impact on limitation of free radicals diffusion in a membrane. In studies focused on interactions with the main blood protein, it was indicated that Pt-3-glu is able to quench natural fluorescence of albumin which is bond with this compound as a result of an impact of hydrogen bonds and van der Waals forces. The measurement results, using TCSPC-FCS method, showed that Pt-3-glu actively interacts with plasmid DNA and causes its condensation.

Conclusions

On the basis of performed research and observed biological activity of a tested anthocyanin, namely petunidin-3-*O*-glucoside, it is concluded that this compound may be used in both pharmaceutical and food industry.

Acknowledgments:

The research was financed by the (Polish) National Science Center, Grant No. 2017/25/N/NZ9/02915, the statutory activities of the Department of Physics and Biophysics, Wroclaw University of Environmental and Life Sciences.

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Effect of immunosuppressive treatment during pregnancy on the metabolism of minerals in the hard tissues of female rats and their offspring

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Background

Organ transplantation is a generally accepted treatment method for organ failure. When successful, it can reverse many complications caused by organ dysfunction, but bone metabolism and mineralisation disorders continue to be problematic. In order to prevent transplant rejection, immunosuppressive therapy is a necessity also for pregnant transplant recipients, which means that the medications may influence foetal development. Calcium, phosphorus and magnesium are some of the essential minerals affecting normal bone formation. 99% of calcium, 85% of phosphorus approx 60% of magnesium in the human body is found in hard tissues. Immunosuppressants may affect on the content of these elements. Therefore in this study, we evaluated the impact of three regimens of immunosuppressive therapy used after renal transplantation on the of the essential minerals (calcium, phosphorus and magnesium) affecting normal bone formation.

Material and Methods

The study was conducted on 32 female Wistar rats. Eight rats comprising the control group were given carrier and olive oil whereas three study groups receiving drug combinations. Each group consisted of eight female rats.

Group 1 (Therapy 1) – receiving CsA (cyclosporine A), MMF (mycophenolate mofetil) and prednisone.

Group 2 (Therapy 2) – receiving Tc (tacrolimus), MMF and prednisone.

Group 3 (Therapy 3) – receiving CsA, everolimus and prednisone.

A total of 148 pups were born: 54 in control group, 36 in group 1, 48 in group 2, and 10 in group 3.

Femur and tooth material collected during necropsy was dried at 100°C until dry mass was obtained. Dry tissue was crushed and 100 mg samples were weighed into plastic vials and labelled. After preparation, the samples were subjected to a microwave decomposition procedure using a microwave digestion system.

The samples were analysed using inductively coupled plasma optical emission spectrometry (ICP-OES) equipped with a concentric nebuliser and a cyclonic spray chamber.

Results

The immunosuppressive regimens under study had no effect on the levels of magnesium and phosphorus, but they did contribute to increased bone calcium levels of mother rats moreover no changes were identified in the levels of the studied minerals in the teeth of mother rats. The

therapy 1 increases bone magnesium levels in the offspring, while a therapy 3 significantly reduces the magnesium level in the teeth of the offspring.

Discussion and conclusions

The immunosuppressive regimens did not affect the levels of magnesium and phosphorus in the rat model (mothers), but they did contribute to an increased bone calcium level. No changes were identified in the levels of the studied minerals in the teeth of mother rats, which may suggest a protective effect of the studied regimens on hard tissues. The use of prednisone, CsA and MMF increases bone magnesium levels in the offspring, while a therapy based on prednisone, CsA and everolimus signifycantly reduces the magnesium level in the teeth of the offspring, potentially affecting mineralisation and strength of hard tissues.

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The expression of virulence- and biofilm-related genes in selected species of uropathogenic Gram-negative rods

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This paper focuses on the overview of virulence- and biofilm-related genes which are present in uropathogenic Gram-negative rods: *Escherichia coli* (UPEC), *Enterobacter cloacae* and *Pseudomonas aeruginosa*. These bacterial species may be responsible for acute, recurrent and chronic urinary tract infections (UTIs), also nosocomial UTIs associated with the use of urinary catheters (CAUTIs) [1,2,4,5].

Uropathogenic strains harbour many genes encoding virulence factors enabling the bacterium to resist and overcome several defence strategies of the host and support different steps in uropathogenesis. The first step in the pathogenesis of UTIs is adhesion (tight and irreversible adherence) of bacteria to host uroepithelial tissue [2]. Virulence factors responsible for microbial attachment include fimbriae/pili belonging to the chaperone-usher family (types: 1, P, S, F1C, F9, 3, UCL, F17/G and IV), amyloid curli fibers, Afa/Dr adhesins, non-fimbrial adhesins belonging to the group of autotrans-

porter proteins (Ag43, Upa), and other afimbrial adhesins (proteins, lipopolysaccharides) [6]. The presence of bacterial cell surface hydrophobicity and T3SS (type III secretion system including thiol-activated pore-forming cytotoxins) or T4SS (type IV secretion system) can be used as a general indicator of bacterial virulence [4]. Also, structures which are primarily associated with other functions may contribute to bacterial adherence, promote adhesion and invasion, e.g.: ability to move (swimming motility) through flagella; some toxins: α-haemolysin, endotoxin, cytotoxic necrotising factors and serine protease autotransporters of the *Enterobacteriaceae* (SPATEs) [2, 4, 5].

After adhesion, uropathogenic Gram-negative rods usually form biofilms consisting of exopolysaccharide- or alginate-surrounded microcolonies, which promote long-term colonisation and persistence in the urinary tract. Pathogens growing in a biofilm mass are a serious threat to human health because of their resistance to immune system factors and antibiotics [2, 4-6].

In uropathogenic bacteria genes encoding virulence and biofilm factors are typically located on horizontally acquired mobile genetic elements (MGE) called pathogenicity islands (PAIs). The organized regulation of gene expression is an important factor in an appropriate invasion, colonization, growth and/or toxin production [1, 3-5].

Nowadays, the objective of researches is to investigate the correlation of bacterial ability to virulence factor expression and biofilm formation with the number of gene copies and their mRNA expression. Uropathogenic bacterial strains are checked for the presence of virulence-related genes and/or biofilm-formation-associated genes using the PCR methods. Total DNA is isolated from overnight bacterial culture using a bacterial genomic DNA purification kit. All PCR and qRT-PCR reactions are performed using *Taq* DNA polymerase. The isolates are screened for the presence of virulence-related genes and genes important for biofilm formation. The sequence coding for 16SrRNA is used as a positive control. PCR amplification products are separated by electrophoresis in agarose gel. Gel images are visualized and analysed [1, 3-5].

The characterization of virulence- and biofilm-related genes can be useful to improve our understanding of the pathogenesis of UTI, to develop effective treatment strategies and to minimize the complications, including kidney failure [1, 3, 6].

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Toxicity of yohimbine – a drug used in erectile dysfunction and body-building supplements

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Introduction

Yohimbine is an alkaloid which is naturally found in barks of the African tree Pausinystalia johimbe and South American Aspidosperma quebracho-blanco tree. The structure of molecule resembles a tryptamine. The most known application of yohimbine is the treatment for impotence. In addition, products containing yohimbine are advertised as a body-building and weight-loss promoting dietary supplements because of its lipolytic effect.

Mechanism of action

The substance is an alpha 2-adrenergic receptor antagonist. The drug-receptor interaction causes an increased release of noradrenaline and dopamine. By blocking the pre- and postsynaptic alpha-2 adrenoceptors, yohimbine dilates blood vessels in skin and genitals. An average oral dose of 5-15 mg produces a therapeutic whole blood level range of 40-400 ng/mL. This effect is used in erectile dysfunction treatment.

Lipolytic effect

Studies have shown that yohimbine has an efficacy in fat lipolysis in the abdominal area. Alpha-2 receptors are found predominantly at the site of resistant fatty tissue. They cause that lipolysis is inhibited, which prevents the oxidation of fat cells and weight loss. In addition, fat tissue accumulates beta-2 receptors that activate the lipolysis and cause the oxidation of fat cells. Yohimbine blocks alpha-2 receptors which results in adrenaline moves towards beta-2 receptors, which increase fat burning. In this case, fat burning begins. To achieve the full potential of yohimbine, it should be used if a person has low body fat but located in resistant places.

Toxicity

- Anxiety,
- Drowsiness,
- Disorientation,
- Tremors,
- Seizures.

Fatal case

An unconscious 23-year-old male body builder was presented to the emergency room with seizures and elevated vitals, and was pronounced dead within hours. Through investigation, jars of yohimbine and caffeine powder were among supplements recovered from the decedent's residence along with arginine, L-carnitine, beta-alanine and testosterone. The decedent had a medical history of low testosterone and hypogonadism. Notable autopsy findings were cardiomegaly (525 g), pulmonary edema and congestion.

Conclusion

Dietary supplements, contrary to pharmaceutical drugs, are not tested for effectiveness nor safety, for example chronic toxicity (as a result of long term exposure). It is especially important in case of yohimbine because it is characterized by a low therapeutic index, meaning there is a small range of therapeutic doses which do not entail dangerous and potentially fatal complications

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Characteristic of bacteriophage phi80-18 infecting *Yersinia enterocolitica*

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Background

Yersinia enterocolitica is a gram-negative bacterium that causes disease called yersiniosis. The infection is manifested as acute diarrhea, mesenteric adenitis, terminal ileitis, and pseudoappendicitis [1]. Most pathogenic for humans is O:3 serotype (occurring mainly in Europe and China) and O:8 serotype (occurring mainly in the USA). The migration of people between continents is taking place without any obstacles that is why the occurrence of *Y. enterocolitica* infections in Europe becoming more frequent. The use of bacteriophages is being considered as an alternative method to control pathogenic bacteria because they characterized exceptional specificity infecting and destroying only the host bacterial cells [2].

Material and Methods

To measure thermal and pH stability bacteriophage phi80-18 was incubated in wide temperature range between 4-80°C and in the pH range between 2-12. The phage titer was evaluated by the double-layer agar method. Bacteriophage phi 80-18 was visualized using a JEOL JEM-1200 EX 80 kV TEM. Phylogeny of *Yersinia* phage phi80-18 was inferred independently using whole genome data and RNA polymerase (RNAP) protein sequence as a phylogenetic marker.

Results

During our research we characterize previously sequenced *Yersinia enterocolitica* bacteriophage phi80-18 showing a wide pH tolerance range and stability at relatively high temperatures. Phylogenetic trees reconstructed using whole genome sequence or RNAP protein sequence confirmes that phi80-18 belongs to *Autographivirinae* subfamily in *Podoviridae*. TEM analysis of viral morphology confirms that phi80-18 belongs to *Podoviridae* family and shows that the estimated virion length is within 70 nm.

Discussion and conclusions

Described properties and wide range of tolerance makes bacteriophage phi80-18 an excellent tool to fight with pathogenic bacteria in the field of biotechnology industry. For example, it can be useful when we think about using phages in food processing industry where bacteriophage biocontrol may become natural method that uses lytic bacteriophages isolated from the environment to specifically target pathogenic bacteria [3].

Research financed by the NCN Sonata Bis 7 UMO-2017/26/E/NZ1/00249

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Optimal conditions for efficient formation of PAMAM dendrimers complexes with 5-fluorouracil

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Background

In today's growing number of cancer cases, more effective methods for fighting cancer are being sought. One possible method is to use a drug carrier to increase the effectiveness of the therapy and eliminate side effects present during pharmacotherapy. In this work poly(amidoamine) (PAMAM) dendrimers were explored as a potential carrier for 5-fluorouracyl, a drug used mainly in the treatment of colorectal cancer [1].

Dendrimers are synthetic polymers with well-defined size and structure. Due to their nano-size, shape and controllability, dendrimers have many applications in biotechnology and seem to be an ideal carrier [2, 3].

Two generations of positively charged dendrimers (4^{th} and 6^{th}) have been studied here, looking for the best conditions for the formation of an effective dendrimer-drug complex by varying molar ratio, ionic strength and pH.

Material and Methods

Experiments were conducted on 4^{th} and 6^{th} generation poly(amidoamine) PAMAM dendrimers with ethylenediamine-based core and terminal NH_2-groups (Dendritech, Inc. Michigan; Midland, MI, USA).

5-Fluorouracil was purchased from Sigma-Aldrich. The solutions were prepared in deionized water and sodium chloride (NaCl).

Analytical techniques were used which allowed the determination of the loading efficiency (UV-Vis spectroscopy), particle size and zeta potential (Dynamic Light Scattering), and the effectiveness of dendrimer and drug adsorption (Quartz Crystal Microbalance with Dissipation monitoring). Additionally the contact angles were determined for the dendrimer layer deposited on the gold sensor (KRUSS).

Results

The complex was prepared under various conditions and the efficiency of binding ligand to dendrimer after dialysis was determined by UV-Vis spectroscopy. The analysis showed that the efficiency of binding ligand to dendrimer is strictly dependent on the conditions of complex formation: molar ratio, ionic strength and pH. Higher efficiency was observed during the application of molar excess of the drug in relation to the dendrimer. As far as other parameters are concerned, the study showed that higher efficiency occurs at lower ionic strength and higher pH.

Using the method of Dynamic Light Scattering, dendrimer particle sizes were determined for both generations. The analysis of the particle size of the complex showed the formation of aggregates during the formation of the complex.

Zeta potential measurement was performed to characterise the dendrimers and used to determine the isoelectric point. The research has shown that zeta potential decreases for higher ionic strength at the same pH, but the isoelectric point remains unchanged.

The QCM-D measurement was using to measure the effectiveness of dendrimer adsorption and the numbers of drug molecules immobilized in the dendrimer structure [2]. Used QCM-D we were looking for the best conditions and the optimal molar ratio for the formation of the complex.

Discussion and conclusions

The results show that poly(amidoamines) dendrimers can be active carriers of 5-fluorouracil for uses in biomedical applications in cancer treatment. Optimal conditions and their effects on loading efficiency for certain parameters have been determined. Future work will be focused on characterisation and optimisation of conditions for formation of dendrimer and drug complexes.

Acknowledgments:

This work was partially supported by project NCN OPUS 2016/23/B/02788, Erasmus+ UK GLASGOW 02

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Effect of Salvianolic Acid B on human gingival fibroblast proliferation

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Background

The anti-oxidative and anti-inflammatory activities of Salvianolic acid B were presented in many *in vitro* and *in vivo* studies. This water-soluble compound was isolated and purified from the crude extract of Salvia miltiorrhiza, the pharmacopoeial plant commonly known in east Asia. Here, we wanted to take a step further and investtigate whether Salvianolic acid B can cause an increased cell proliferation as well. To check this possibility, human primary gingival fibroblast cell line was pre-treated with Salvianolic acid B in six different, grading concentrations. Cell viability was analysed via the MTT assay after three different times of incubation with compound. Additionally, the expression of collagen III protein after Salvianolic acid B incubation was assessed by immunocytochemical method.

Material and Methods

Human gingival fibroblasts were isolated from a patient. As the main method we used MTT assay after 24, 48 and 72 hours incubation to investigate cell proliferation. Salvianolic acid B tested concentrations were: 25 µg/ml, 50 µg/ml, 75 µg/ml, 100

µg/ml, 150 µg/ml and 200 µg/ml. To observe collagen III expression changes we performed immunocytochemistry assay using rabbit polyclonal collagen III antibody (ab7778).

Results

The MTT assay showed that Salvianolic acid B has a stimulating impact on human fibroblast proliferation. There is a correlation between compound concentration and amount of newly formed cells.

Discussion and conclusions

The results are promising and can lead to further clinical research on, inter alia, wound healing process.

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Pneumocystis pneumonia – who should be vigilant?

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This paper reviews the risk of infection of an opportunistic pulmonary fungus – *Pneumocystis jirovecii* – in different groups of patients. Immunocompromised individuals constitute the main group of risk of infection and development of symptoms of *Pneumocystis* pneumonia (PCP, or pneumocystosis). PCP used to be the most common AIDS-defining infection in 1980s, however, due to the introduction of anti-retrovirus therapy, its incidence in HIV-infected individuals has significantly decreased since then. Nowadays, patients with immunosuppression caused by other factors than HIV infection are considered the main group of risk, such as e.g. transplant recipients receiving anti-rejection drugs, cancer patients undergoing chemotherapy, patients with inflammatory and rheumatic diseases, as well as preterm infants. In these cases, the symptoms of PcP (unproductive cough, low-grade fever, progressive dyspnoea) are usually characterized by rapid onset, faster progression and poorer prognosis as compared to HIV-patients. Moreover, even very low intensity of infection may be sufficient to cause symptoms of PcP, which can even lead to death. Therefore, it is extremely important to develop sensitive and specific diagnostic methods, in order to detect even the low level of the pathogen in patients' samples and introduce specific therapy prior to the development of this serious disease.

In turn, the immune system of healthy individuals usually eliminates the infection quickly, however, *Pneumocystis* may persist in lungs in the asymptomatic form. Since the transmission of this pathogen occurs via the airborne route, such carriers serve as a source of infection in the human population, posing a risk for immunocompromised individuals. Therefore, colonization, although asymptomatic, is also an important epidemiological issue. Furthermore, even individuals without immunosuppression,

such as patients with various pulmonary diseases, can be more susceptible to infection due to specific factors.

The present review focuses on different factors affecting the risk of *P. jirovecii* infection and PcP development, including type of underlying disease, drugs used, co-infections and comorbidities.

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Interleukin 13 as an immunomodulator in various disease

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There is a growing evidence that chronic inflammation may play a key role in thepathogenesis and development of various diseases, including cancer. IL-13 is a pleiotropic cytokine with anti-inflammatory and immunoregulatory activity [1].

Literature data indicates its role in the pathogenesis of cancer, such as breast cancer, ovarian cancer, pancreatic cancer, colorectal cancer, head and neck cancer, bladder cancer and lymphoma cancers. However, the results of some studies indicate a contradictory role of IL-13 in promoting and fighting the progression of cancer. The involvement of IL-13 in the escape of tumor cells from host immune surveillance is important [2, 3].

In addition, IL-13 is found to be involved in other disease such as parasitic infections, asthma, atopy, nephrotic syndrome, gastrointestinal tract diseases, and arthritis [4].

Further research, especially on the role of pro-cancer or anti-cancer IL-13 will constitute an important prognostic and/or diagnostic aspect. The use of IL-13 inhibitors in targeted immunotherapy is also being considered [5, 6].

The aim of this work was to analyze literature data on the importance of IL-13 as an anti-inflammatory immunomodulator in various disease states and potential mechanisms of its action.

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Analysis of interleukin 13 and angiogenin in patients with bladder cancer

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Background
Participation interleukin 13 (IL-13) in the process of carcinogenesis was well studied, but we have only few reports on its involvement in bladder cancer (BC). Angiogenesis (formation of new blood vessels), plays a key role in the process of tumour growth and metastasis. It enables the delivery of nutrients and oxygen to cancer cells. The aim of the study was to investigate the role of IL-13 as an anti-inflammatory immunomodulator and angiogenin (ANG) as a stimulator of the angiogenesis process in patients with BC.

Material and Methods
The concentration of IL-13 and ANG in the plasma of BC patients and healthy controls were measured by enzyme-linked immunosorbent assay. These parameters were examined in the whole group of BC patients and in subgroups depending on clinical stage: non-muscle invasive bladder cancer (NMIBC), muscle invasive bladder cancer (MIBC), histopathologic malignancy low grade (LG), high grade (HG) and in primary and recurrent BC. To assess the IL-13 and ANG diagnostic value, ROC curves were plotted, cut-off points as well as sensitivity and specificity were calculated. The research was approved by the Bioethics Committee No. (KB-292/2-16).

Results
In patients with bladder cancer were found significantly higher mean plasma concentration of IL-13 ($p<0.001$) and ANG ($p<0.001$) compared to the control group. Higher mean IL-13 plasma concentrations corresponded to lower disease stages (NMIBC, LG). In contrast, mean ANG levels were higher in advanced stages (MIBC, HG) of BC. The mean concentration IL-13 and ANG were similar in primary cancer and recurrence BC.

Discussion and conclusions
Higher IL-13 expression in bladder cancer tissues has been demonstrated by immunohistochemical studies. Urinary levels of IL-13 have also been shown to be useful as a marker in bladder cancer. Higher serum levels of ANG have been demonstrated in many types of cancers.

In the above study, the potential of the tested indicators as diagnostic parameters in bladder cancer has been demonstrated.

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Theoretical studies on the structure of fagopyrin

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Background

Compounds with a double anthrone moiety find an use in the treatment of cancer, depression, as a natural laxative and against constipation. One of the double anthrone is fagopyrin, a natural ingredient of *Fagopyrum esculentum*. Fagopyrin occurs as many conformers. (Fig. 1) Physical and therapeutic properties of fagopyrin result from its molecular structure that is not determined. Theoretical methods were used to determine the geometrical and electronic structure of fagopyrin conformers.

Material and Methods

Structures of fagopyrin were optimized using Gaussian 16 [1] software. BLYP/6-311++G(d,p)-D3 method was applied to optimization in the gas phase. QTAIM analysis was performed using AIMALL [2] program. Non-covalent interactions were described by NCI [3] software.

Results

Figure 1 presents the molecular structure of fagopyrin and the substituents present in the plant material. For fagopyrin A - F and different orientation of hydroxyl group the structure has been optimized and many conformers characterized by different energy have been found. Theoretical methods allowed to determine the intramolecular interactions in the fagopyrin derivatives. QTAIM and NCI analysis indicates the strong OH···O hydrogen bonding in the anthrone moiety. The presence of substituents containing nitrogen atom allows formation of OH···N hydrogen bond linking hydroxyl group in the anthrone moiety and the nitrogen atom in the cyclic substituent.

Discussion and conclusions

The system of the strong O-H···O···H-O hydrogen bonds in the anthrone molecule can be changed under formation of O-H···N hydrogen bond to the nitrogen substituent. Specific properties and numerous application of a double anthrone compounds result from their molecular and electronic structure. Molecular structure of fagopyrin is unexplored thus, the use of theoretical methods allows to approximate its structure that determines physicochemical properties.

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	R1	R2	R3	R4
Fagopyrin A	CH_3	CH_3		
Fagopyrin B	H	H		
Fagopyrin C	CH_3	H		
Fagopyrin D	CH_3	H		
Fagopyrin E	CH_3	CH_3		
Fagopyrin F	CH_3	CH_3		

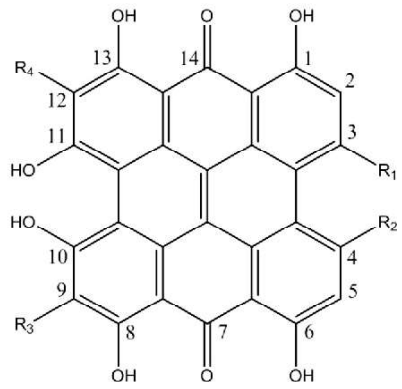

Figure 1. Possible conformers of fagopyrin [4]

Theoretical study of monoanthrones

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Background

Monoanthrones are tricyclic compounds of plant origin. Due to their wide biological activity, they are used in both herbal medicine and medicine. An interesting feature of monoanthrones is their non-planar structure. The therapeutic properties of monoanthrones result from the geometrical and electronic structure. For better understanding of the mechanism of drug action, the effect of substitution in the aliphatic ring and in the side ring on the geometry has been studied.

Material and Methods

Optimization of the geometry of several series of compounds with different substituents in the rings was carried out. The investigated molecules were optimized using a Gaussian 16 package at DFT B3LYP/6-311++G** level, which including Grimme dispersion. Calculations of electron density were performed using the AIMALL program. Aromaticity expressed by the HOMA (Harmonic Oscillator Model of Aromaticity) parameter for anthrone aromatic and aliphatic rings were determined.

Results

The change of the angle between the anthrone aromatic rings is associated with the change in electron density. The central, aliphatic ring takes on a partly aromatic character after substitution of the side ring with an electron donating substituent, while substitution of the side ring with an electron withdrawing groups causes losing partly aromatic character.

Discussion and conclusions

Aromaticity of the anthrone rings is affected by the electron donating and electron withdrawing properties and size of the substituent linked to the aromatic side ring as well as to the central aliphatic ring.

Substituents in the aromatic ring affect the geometry and electronic structure of the central ring.

Substitution in the central ring have the greatest impact on the structure of the entire molecule.

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The spectroscopic studies of the interaction between human apo-transferrin and copper(II) complexes based on 2,9-dimethyl-1,10-phenanthroline and 1,3,5-triaza-7-phosphaadamantane-7-oxide

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Background

Spectroscopic studies involving transition metal complexes and blood proteins such as human serum transferrin/albumin are essential for the understanding of the biological activity of the drugs in terms of nature and strength of the interactions [1, 2]. Such interactions may affect the concentration and deactivation of the drug and thereby influence its availability and toxicity during chemotherapy [3]. For example, when cisplatin is introduced into the body intravenously, 50−61% of platinum is bound to HSA [4,5] and the binding is essentially irreversible with less than 5% loss of bound-platinum after extensive dialysis. It is very important from the pharmacological point of view, because only unbounded with protein part of the drug is biologically active. In this context the subject of our research is the analysis of the interactions between biologically active copper (II) complexes and human serum apo-transferrin.

Material and Methods

All compounds (1, 2, 3) were synthesized by prof. Piotr Smoleński and co-workers from Faculty of Chemistry University of Wrocław.

Apo-transferrin (≥ 98%, Sigma-Aldrich) was used without prior purification. The final solutions were prepared in PBS (pH 7.40) in the molar ratios (protein):(drug) = 1:0–1:16 with the protein concentration equal 2 μM. Samples were incubated at 300 and 310 K for 5 min.

Emission fluorescence spectra were recorded on Jasco 8200 spectrofluorimeter in the range of 300–500 nm using 1.0 cm quartz cells and the λex = 280. All fluorescence intensities were corrected for the inner filter and dilution effects and the corrected values were used to determine the quenching mechanism and binding data. Moreover, the copper complexes showed a fluorescence signal in the measured range. Therefore, all spectra are shown as different spectra of (apo-Tf-copper complex)-(copper complex).

Circular dichroism measurements were carried out on a Jasco J-715 spectropolarimeter in the range of 190-250 nm using 0.1-cm cuvettes.

Results

Under physiological conditions titration of the protein with small amounts of the complexes caused distinct decrease in fluorescence intensity of the protein and the maximum band position was red shifted ca. 5 nm. It indicates that the apo-Tf conformation was changed and the protein's chromophores were moved to a more polar environment. Moreover, based on the Stern – Volmer equation it was shown, that protein fluorescence quenching by 1/2 complexes was initiated by both static and dynamic processes and it was static process in the case of 3.

Under tested conditions only one binding site (n) in protein for all copper complexes exists. The association constants (Ka) decreased with the temperature increases for 1 and 2, suggesting formation of the unstable complexes. The binding constant of apo-Tf – 3 system increased with temperature increase, indicating the formation of the stable adduct and endothermic process.

The interaction of 1 and 2 with apo-Tf had an insignificant effect on its secondary structure and, upon binding, the complexes α-helix content decreased about 1-2%, respectively, when the molar ratio apo-Tf:1/2 was 1:20. In contrast to 1 and 2, binding 3 complex to apo-transferrin caused extensive changes in conformation of the protein reducing α-helix content about 12% at the molar ratio apo-Tf:3 equal 1:20.

Discussion and conclusions

All tested compounds interact with human apo-transferrin, causing a conformational changes of the protein. Complex 3 showed the most extensive interaction with the loss of helical stability of the protein. The positive values of ΔS^0 and negative ΔH^0 for apo-Tf –1/2 systems indicated electrostatic interactions, and both positive parameters for 3 revealed hydrophobic and ionic interactions. Moreover, all reactions between copper complexes and human apo-transferrin were spontaneous processes.

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Persistent organic pollutants: impact on the incidence of type 2 diabetes

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Persistent organic pollutants (POPs) are organic chemicals that are toxic and highly resistant to any kind of environmental degradation. Most of them are currently or were in the past produced as pesticides, insecticides, herbicides, solvents, flame retardants, and as a chemical intermediates. Also generated unintentionally during combustion, thermal and industrial processes. Although a lot of these compounds have been banned or placed under international control, POPs are still common in the environment and

accumulate in soil, sediments, and the food chain. People are still exposed to the toxins mostly through their diet, particularly fatty animal food, such as meat, fish, and dairy products.

The number of studies have reported potential associations between this exposure to POPs and various harmful health effects, including cancers, increased birth defects, neuroendocrine disruption, reproductive problems, and metabolic disorders, like obesity and diabetes type 2 [1,5,6]. The aim of this paper was to assess the impact of POP exposure on the incidence of type 2 diabetes, based on a review of the current literature.

Numerous epidemiological and cross-sectional studies indicate a correlation between POP serum level and the development of type 2 diabetes [1, 2, 4-6]. It points to certain polychlorinated biphenyls (PCBs) and several organochlorine pesticides (OCPs), in particular dichlorodiphenyltrichloroethane (DDT) and dichloro-diphenyldichloroethylene (DDE), as potentially adversely affecting substances [1, 4]. Due to their high lipophilicity and persistence in the body, the POP serum level increase with age. However, the POP serum levels varies a lot between studies and the results are inconsistent [1, 4-6]. Differences in study population, POP exposure distributions, varying methodology, presentation and analysis of the results could partly explain these discrepancies [1, 3-5]. Thus, the lowest published toxic dose (TD_{LO}) of individual POP compounds remains uncertain.

The pathophysiology mechanism underlying between POP exposure and development of diabetes also seems to be complex. Some researchers suggest that POPs are associated with insulin resistance, impaired glucose uptake, metabolic syndrome and abdominal obesity [2, 3]. Other studies indicate on beta cell function impairment [1, 6]. Unfortunately, the long period between exposure and development of health problems, the influence of a range of other environmental factors and possibly additive effects of the POP compound mixture hamper investigation [3,5].

In conclusion, numerous studies reported a strong correlation between serum concentration of POPs (especially organochlorine compounds) and diabetes. However, there is a strong need for further animal and *in vitro* research to clarify the impact of POPs on the incidence of type 2 diabetes. In the face of the rapid increase in diabetes prevalence and highly developed industry, understanding the role of environmental chemicals like POPs in the development of diabetes is an emerging issue.

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Edible Insects as Bread Making Ingredients

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The use of edible insects as a protein source in food stretches back for millennia, however the practice has yet to win over European and Western Consumers, industry regulators, and sanitary regulators. Furthermore, the economic viability and environmental impacts of the harvest of insects has yet to be proven. Within the laboratory, the use of edible insect flour in both conventional wheat-based flour bread and gluten free bread has shown favourable results in overall protein, fat, and structural effects on the nutritional content of these types of bread.

The overall water absorption capacity was shown to be reduced when using insect flours ranging from 45-57% along with fat contents from 27%-36% [1]. However the bread making process could be carried out with all composite flours showing similar texture and volume parameters when compared to conventional wheat bread, yet with higher protein and fibre concentrations attributed to insect flours.

Results confirm that the enrichment with cricket powder can lead to the production of gluten free bread with acceptable technological properties and high protein content [2]. Tests have proven that the porous structure which is the signature of conventional wheat bread can be reproduced using a gluten-free batter containing insect-derived flour. The porous structure can be attributed to the insect flour's protein and lipid contribution to the mixture.

When sensory and overall cultural acceptance is taken into consideration, a bread with 10% cricket flour showed a global liking by untrained panellists [3].

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Functional properties of blends of rice and house cricket (*Acheta domesticus*) flours

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Background

The demand for gluten-free products has been rising as the total number of consumers avoiding wheat/gluten-containing products increases exponentially [1]. Meanwhile, the main problem of gluten-free breads is the fact that they are overloaded with structuring agents, the main purpose of which is to create the spongy/porous structure, found in typical - wheat breads, from ingredients which do not contain the gluten-protein [1]. The predominant ingredient in a gluten-free formula is starch, while native flours are few. The more frequently used flour is rice flour, which is rich in starch, but has an insufficient protein level. Edible insects are a good source of protein and can also be used as nutritional enrichment in gluten-free formulations.

The main objective of this study was to investigate the hydration properties of rice flour: powdered house cricket blends in order to assess its suitability as gluten-free baking ingredient.

Material and Methods

Hydration properties like water holding capacity (WHC), oil absorption capacity (OAC), water solubility index (WSI) along with the water absorption index (WAI) in gluten-free flour mixtures composed of varied blends of rice flour and house cricket flour were studied. Both flours where purchased commercially on the local market (Wrocław, Poland) and six mixtures were prepared to contain 5%, 10%, 15%, 20%, 25%, and 30% of cricket flour, along with two controls of 100% rice and 100% cricket flours.

Results

The results revealed a very interesting behaviour of cricket flour. The water holding capacity in cricket flour sample alone was significantly higher than in rice flour one (2.48 g/g d.b. vs. 3.02 g/g d.b.), but its impact in the studied blends was not significantly different. Also, the water soluble index of cricket flour was the highest among the studied samples, while the water absorption index was the lowest, showing different water maintaining behaviour after heating. Furthermore, the water solubility index of rice flour was not impacted by cricket flour addition. Furthermore, OAC was shown to be higher using the 100% cricket flour control as compared to the 100% rice flour control. Thus a gradual increase of oil absorption capacity was observed starting at an average of 1.478057316 g/g d.b at a 5% Cricket blend, to an average of 1.507978905 g/g d.b at a 30% Cricket blend.

Discussion and conclusions

Powdered house cricket can be applied as baking ingredient because of its high nutriational value. As baking ingredient, especially in gluten-free bread-making, the specific water hydration properties make powdered house cricket a demanding raw material. Hydrophilic and hydrophobic interactions among bread ingredients result in batter structure formation and further in crumb porosity of resulting bread. High protein and fat content of *Acheta domesticus* powder revealed more hydrophobic behaviour while chitin acting like fibre absorbs water.

The obtained results open very promising field for successful application of house cricket powder as valuable ingredient in gluten-free breadmaking and promising perspective as contributor of important nutrients.

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Connection between laterality and primary reflexes, balance, sensory profile in group of children from 4 to 7 years old

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Background

Laterality is functional domination of one cerebral hemisphere. Any dysfunction of cerebral hemispheres work could involve problems with balance, concentration and psychomotor activity. The aim of the study is to verify is it any connection between type of laterality and primary reflex integration, balance and sensorimotor profile.

Material and Methods

Fourty nine healthy children aged 4-7 years were examined. In this group was define the type of laterality of hand, leg, eye and ear. In each category children were divided into two groups – left and right laterality. Their primitive reflexes were investigated by S. Goddard's test. During the research time of stand on one leg (to check the balance) was measured and sensorimotor profile was also defined.

Results

Group of children with dominance of left leg have more problems with control their preferred leg and also more difficulties with keeping stable position at one leg stand test. That makes they stand shorter at dominant (left) leg than children with dominance of right leg. In group of left-leg children is also more active forward tonic labyrinthine reflex (TLR). Children with dominance of left eye have more symptoms of dyspraxia than group with right-eye children. In this group is also high activity of TLR in extension. In group with dominance of left hand most of children have auditory hyperactivity. It is also very common in group of children with crossed laterality. There are no important differences between examined group of children with left and right dominance of ear.

Discussion and conclusions

Research shows correlation between laterality and activity of primitive reflexes, balance and sensorimotor profile. There is worse level of maturity of nervous system in examined group of children with left side domination and crossed laterality.

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Smart behaviour of materials used for controlled delivery of bioactive agents

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Numerous limitations connected with traditional chemotherapy leads to the development of the novel concept of Smart Drug Delivery Systems (SDDSs). It allows to overcome barriers of conventional treatments, such as uncontrollable release of drugs, nonspecific distribution, rapid clearance or low bioavailability. Moreover, smart nanocarriers make possible to deliver the bioactive agents to target sites and what is crucial, the drug release rate can be controlled over time [1, 2].

Nanoscopic drug delivery systems are colloidal particles of size less than 500 nm, which possess a high surface area to the volume ratio. They are characterised by improved effectiveness through usage the pathological physiology of the tumor tissue environment i.e. angiogenesis, hypervascularization of vessels feeding the tumor or reduced pH. Furthermore, there is better nanocarrier accumulation at tumor tissues due to the enhanced permeability and retention (EPR) effect [1, 2].

Currently, many studies in nanomedicine focus on controlled drug release systems, that are sensitive to different types of triggers, what define them as smart or stimuli-responsive materials. They possess the ability to respond endogenous or exogenous stimuli including: pH, temperature, enzyme concentration, magnetic field, light, ultrasound, ionic strength or glucose. These kinds of materials are utilized to control the kinetics of drug release at the specific place and at the given time [3].

Temperature is one of the most carefully studied triggers used in drug delivery in cancer therapies. Many polymers are completely soluble below a certain temperature referred to as Lower Critical Solution Temperature (LCST), whereas above this point polymer changes its structure and precipitate from the solution. This kind of phase change can be used for controlled destabilization of polymeric structure and effective drug delivery [4].

In this research, different kinds of smart behaviour of materials are presented. The kinetic control of drug release possesses many benefits for patient health including delivery drug to the specific place with the reduction of dosage frequencies (the drug concentration in target sites is constant for longer time). Due to the number of advantages, smart nanocarriers are currently very promising and intensively studied topic.

Acknowledgements

The support of statutory activity subsidy from the Polish Ministry of Science and Higher Education for the Faculty of Chemistry of Wroclaw University of Science and Technology is gratefully acknowledged.

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The expression of 'major housekeeping' genes decreases in stress respons – is phosphorylation involved?

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This work was funded by the Opus 11 grant from the National Science Centre, Poland no. O-2016/21/B/NZ4/00541.

Background

The process of transcription is one of the key adaptive mechanisms and needs to be strictly controlled in response to environmental factors and stimuli (as heat shock). Recently, interest in this topic has been growing among scientists, because it is still not known much about the mechanisms controlling it.

Heat shock is an invaluable model for studying mechanisms regulating gene expression and is well known and easy to control [1]. Many papers report that during stress, transcription is shut down globally while only a few loci are highly activated [2]. These active loci are connected with heat shock proteins (Hsp) family which functions as intra-cellular chaperones.

Lamins are evolutionarily conserved proteins classified as type V intermediate filaments, which are involved in the regulation of gene expression, chromatin organization, DNA replication and repair, signaling, developmental regulation, and nuclear positioning [3]. In order to play such a variety of functions, lamins interact with many different nuclear proteins, which are directly or indirectly responsible for a particular function. Lamins (the main component of the nuclear envelope) together with associated proteins built a complicated platform for the regulation of nuclear processes. It has been proved that the chromatin regions located near the nuclear envelope consist mainly of heterochromatin – transcriptionally inactive regions.

Our research suggest that lamins and associated/interacting proteins are significantly connected with transcription regulation. In this work, we focus on changes in gene expression profile in stress response. Our results also suggest that the phosphorylation status of HSF and lamins changes. Does the phosphorylation cause the transcription shut down or is it the result of it?

Material and Methods

Cell culture and heat shock treatment

All experiments were performed on *D. melanogaster* embryonic cell line – Kc. Cells were maintained in suspension culture (in Schneider`s Drosophila Medium from Gibco with 10% FBS and 1% antibiotic-antimycotic) at 23°C as normal conditions. To induce the heat shock cells were incubated at 37°C for 1 h before further experiments.

Real-time quantitative PCR and data analysis

Cells were lysed on plates and total RNA was extracted then the cDNA synthesis was performed. RNA extractions and cDNA synthesis from all samples were performed for three biological replicates.

RT-qPCR was performed using QuantStudio™ 5 thermocycler and data were calculated by connected Applied Biosystems™ qPCR analysis module.

Western blot/Immunofluorescence and analysis

Standard western blot/immunofluorescence procedure was performed and data were analyzed using Image Lab/ImageJ software.

Results

We have developed a protocol that allows us to study the stress response in cells.

We have found that in response to stress, there is a decrease in the expression of transcripts encoding key proteins for cell functioning, such as actin, tubulin, lamins and topoisomerase II. In contrast, the protein level remains stable.

Our data show that ribosomal RNA and transcripts for ribosomal proteins are the most stable and perform well as the reference for studying stress response.

We have shown that under stress the phosphorylation of HSF and lamin Dm occur. We also have shown one of a stress-dependent phosphorylation site in lamin Dm – Ser25.

Discussion and conclusions

Epigenetics is a field of the future. So far, many chromatin remodelers with histones and RNA polymerase II in front have been identified as those in which post-translational modifications either activate or lead to gene repression. Global transcription shut down is clearly visible in heat shock. In this study, we show that under stress, transcripts level for genes encoding for the key proteins of the cell decreases e.g. lamins, topoisomerase II, tubulin and actin. These transcripts are often used as stable references. Here we prove that ribosomal RNA-related controls work better.

In contrast to data from gene expression, protein levels remain stable in stress. However, the phosphorylation status of proteins is different.

In our project, we show that during heat shock, specific phosphorylation of lamin occurs on Ser25, which results in a change in its solubility and potentially leads to stronger binding of chromatin in stress. These data may indicate that lamins play a key role in turning down gene transcription.

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Colorimetric detection of amino acid sarcosine in urine: influence of zinc (II) ions

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Backround

The most common cancer in men is prostate carcinoma. According to WHO statistics, over 1.1 million new cases are diagnosed worldwide every year. Suitable tumor markers are sought for early diagnostics. One of the biochemical candidates is the amino acid sarcosine (SAR). There is an increase in sarcosine levels in the urine. In several published studies, sarcosine levels ranged from 1 to 25 μM. The aim of this work was to optimize the detection of sarcosine in artificial urine.

Material and methods

All chemicals used for this analysis were purchased from Merck (Germany). Used artificial urines were (15 types): AU-N, AU-Pro-1, AU-Pro-2, AU-keto-2, AU-pH-1, AU-pH-2, AU-Hemog-1, AU- 1-Brown, AU-2-Opalko, AU-4-Grases, AU-6-Christmas, AU-3-Bact, AU-5-Mayrovitz, AU-7-Chutipongtande. The measurement was performed on a pre-washed polystyrene plate (HydroFlex) using a photometer (TECAN, Switzerland). The total sample volume was 250 µl and the substrate solution volume was 750 µl. The substrate solution contained 4-AAP (4-aminoantipyrine), sarcosine oxidase (SOX), horseradish peroxidase in 0.2 M phosphate buffer (pH 8). Electrochemical analysis of zinc: Zinc chloride was dissolved in phosphate buffer pH 8. The zinc concentration was determined in acetate buffer pH 5. Three-electrode setup (3M Ag / AgCl / KCl reference electrode, glass-carbon auxiliary electrode, HMDE working electrode) was used. Then, the zinc (II) ion solution was added to the substrate solution and measured with the urine sample (25°C). All reactions were documented in the form of photographs. The color reaction was evaluated and statistically processed in the Laboratory Information System Qinslab (Prevention medicals, Czech Republic).

Results and discussion

The Trinder reaction is based on the use of a suitable dye (such as 4-aminoantipyrine) in the presence of hydrogen peroxide and peroxidase. Sarcosine oxidase is a flavor-enzyme that is involved in the oxidative demethylation of sarcosine, and its reaction with water and oxygen reduces sarcosine to glycine, formaldehyde and hydrogen peroxide.

The Trinder reaction produces a quinone imine dye which is photometrically evaluated. The test without zinc ions was performed with sarcosine additions (216, 166, 125, 62.5, 31.2, 15.6 and 0 µM) in each of the modified urines (n = 15). Sensitivity in all visually evaluated urines (15 types) ranged from 0.78 to 1; specificity always corresponded to 1, AUC (area under curve) ranging from 0.89 to 1. LOD values ranged from 2 to 73 µM in all urine types; LOQ values ranged from 9 to 195 µM. Subsequently, zinc ions (15 mM) were added to the reaction. Reaction conditions and sarcosine concentration remained the same. Resulting values of reaction with zinc ions (visual evaluation) always corresponded to sensitivity 1; specificity 1, AUC also around 1. LOD values ranged from 4 to 53 µM in all urine types; LOQ values ranged from 13 to 174 µM. In both reactions (with and without ions) the reaction rate was measured. The Michaelis Menten constant (according to the Eadie-Scatchard and Lineweaver-Burk method) were twice as high (4.53) for the zinc ion reaction than for the zinc ion-free reaction (2.52).

Conclusion

Zinc ions in these urines have been shown to support the reaction by up to 100% compared to the zinc-free group.

Acknowledgements

The work was realized with the support of project SarkoTest 165/2015 and The European Technology Platform for Nanomedicine.

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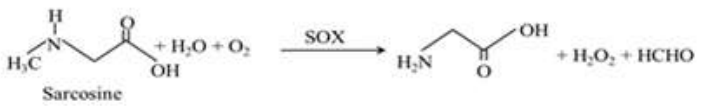

Sarcosine

Glymphatic system in pathophysiology of the central nervous system

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Background

Glymphatic system plays a vital part in maintaining homeostasis in the central nervous system. It not only delivers vital substrates to the brain parenchyma but also clears the parenchyma from unnecessary metabolites. This paper focuses on the role of glymphatic system in pathophysiology of the central nervous system and the potential usage of the glympathic system in clinical management.

Material and Methods

Authors used Google Scholar and Pubmed databases to gather the most recent and reliable sources. Following key-phrases were used: glymphatic system, pathophysiology, central nervous system, injury. Illustrations were prepared by the authors using computer programs: Gimp, Inkscape, LibreOffice Impress

Results

Authors have selected 6 number of articles which best describe recent findings and are most relevant to the possibility of clinical application.

Discussion and conclusions

As most studies on the glymphatic system are based only on animal models, the value of discussed findings is limited, especially in regard to potential clinical applications. However, the research carried out so far allows us to see the potential for a better understanding of the mechanisms of the glymphatic system.

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Usefulness of ultraviolet-induced spectral bone fluorescence in assessing the time of inhumation

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Background

Estimating the time since death of an individual (PMI – Post Mortem Interval) is very important for forensics, as well as for archeology and anthropology. Determining PMI requires an interdisciplinary approach. Therefore, creating a simple, fast and effective method of estimating the time since death can revolutionize both archeology and physical anthropology, but also forensic sciences.

The chemical composition of bones changes with the time they remain in the ground. Differences in bone autofluorescence excited by ultraviolet radiation can be used to determine the approximate period of their inhumation [1, 3]. This is indicated by the results of studies carried out in the past by various researchers, but these results are not unambiguous, especially they used different methods of sample preparation and measurement of bone autofluorescence.

The aim of the study was therefore to check whether it is possible to determine the time of inhumation of human remains using spectral analysis of bone fluorescence excited by ultraviolet radiation.

Material and Methods

Human bones used for measurements have been powdered in a mortar. They were small fragments – about 0.5 cm in size taken from the ribs. The bones came from various periods from the Neolithic to the 16th - 18th century, they were obtained from the skeletal collection of the Department of Anthropology Polish Academy of Sciences.

Powdered bones were suspended in 0.9% NaCl solution in a ratio of 1 mg bones: 1ml NaCl solution. Measurements were made in Department of Physics and Biophysics by spectrofluorimetric method over a wide range of fluorescence excitation wavelengths.

Results

The most interesting results were obtained in the case of bone fluorescence excitation with 370 and 380 nm wavelengths. The obtained results are presented in figure 1 and figure 2.

Differences in fluorescence intensity of individual samples are clearly visible, but they are not correlated with the chronology of the periods from which the bones come.

Discussion and conclusions

Previous studies on dating by bone autofluorescence also show no relationship between fluorescence intensity and sample age [2, 3]. Instead, they indicate a relationship between fluorescence intensity and collagen content in the bone sample. Due to the varied environmental conditions in which the bones were found, the amount of collagen retained and its degradation products may differ significantly even between samples from the same period [4]. For this reason, based on the analysis of available literature and the presented experiment, the only valid conclusion is to carry out more detailed analyzes on a much larger amount of material.

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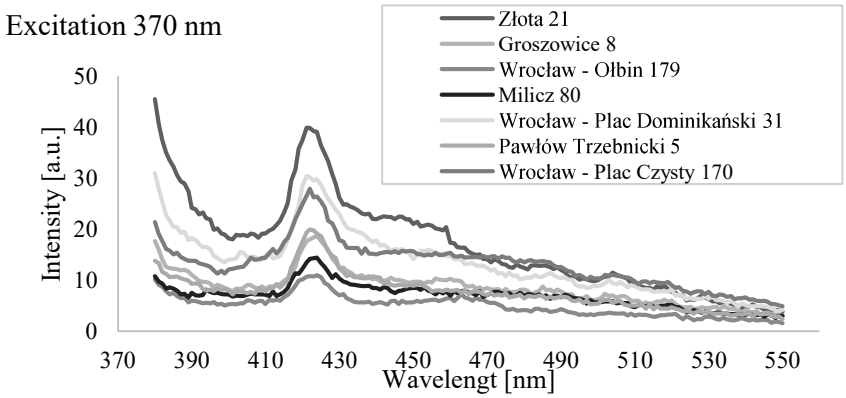

Figure 1 Emission spectrum of bone's probes with 370 nm excitation wavelength

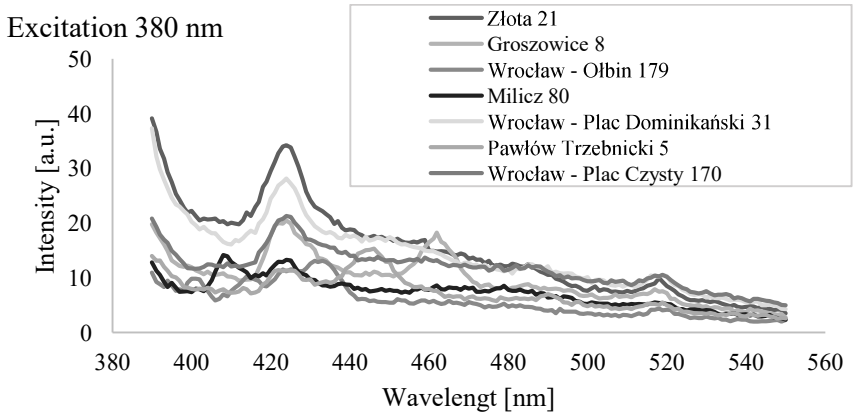

Figure 2 Emission spectrum of bone's probes with 380 nm excitation wavelength

Sensitive analysis of sarcosine in artificial and real urine after derivatization with ninhydrin

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Background

Sarcosine is an amino acid that is studied as a potential marker of prostate cancer and other diseases. It is commonly found in muscle, other body tissues and fluids. HPLC with derivatization is commonly used for sarcosine detection. The aim of this work was to design a simple procedure for sarcosine analysis in real urine. Sarcosine was

determined by ion exchange liquid chromatography with ninhydrin derivatization.

Material and Methods

The AAA500 analyzer from Ingos (Prague, Czech Republic) was used for the analyzes. The chemicals hydrindantine, thiodiglycol, methylcelsolve and 4 M acetate buffer were from Ingos (Prague, Czech Republic). Buffer chemicals (mobile phase) such as citric acid and sodium citrate were from Lachner (Neratovice, Czech Republic). Ninhydrin, sodium chloride and other chemicals were from Sigma-Aldrich (St. Louis, USA). The distilled water was prepared by the Aqual system (Tisnov, Czech Republic) and the ultrapure water was prepared by the ELGA system (High Wycombe, United Kingdom) up to 18 MΩ. The pH control was performed using a pH meter (VWR, USA).

Results and Discussion

It is known that urine is a complex matrix of ions, sugars, peptides, proteins and other substances. In addition to the significant qualitative variability is considerable variability uantitative. Amino acids need to be derivatized to detect them. A suitable derivatizing agent is ninhydrin. The separated amino acids react with ninhydrin. The detection solution was prepared from 11.2 mM ninhydrin, 75% methylcellosolve and 1 M acetate buffer. Hydrindantine was used as reducing agent. The resulting product was detected by a two-channel detector at 440 and 570 nm. The separation was carried out in a glass column with an ion exchanger having a particle size of 8 μm. The elution phase contained 58 mM citric acid, 16 mM sodium citrate, 158 mM sodium chloride and 0.25% thiodiglycol. The mobile phase flow rate was 0.25 ml/min and the ninhydrin flow rate was 0.2 ml / min. The reactor temperature was 131°C. The sample injection volume was 200 μl. The optimization steps included an experiment with samples in water, buffer, artificial urine and real urine. When the sarcosine standard in ultrapure water was used for the assay, LOD 1.7 μM and LOQ 5.7 μM were detected. When the standard was prepared in dilution buffer (72.9 mM citric acid, 196.8 mM sodium chloride and 0.5% thiodiglycol) the limits were lower (LOD 0.5 μM and LOQ 1.8 μM). Using artificial urine (Chutipongtande), measurements were performed at pH 6 (untreated solution) and at pH 2. At pH 6, LOD was 6.0 μM and LOQ was 19.7 μM, but at pH 2 there was a slight reduction in the limits to LOD 3.9 μM and LOQ 12.9 μM. The results indicate a need to adjust the sample to a low pH. The pH 2 was chosen according to the elution buffer, which has a pH of about 2-2.2. In mixed urine, urine samples were adjusted to pH 2.0; 2.1 and 2.2. Sarcosine calibration at concentrations of 62.5; 31.3; 15.6; 7.8; 3.9 and 0 was performed using mixed urine. At pH 2.0, the lowest sarcosine detectable concentration was about 7.8 μM, LOD was 14.2 μM and LOQ was 46.8 μM. At pH 2.1, the lowest sarcosine detectable concentration was about 3.9 μM, LOD was 10.9 μM and LOQ was 35.9 μM. At pH 2.2, the lowest sarcosine detectable concentration was 15.6 μM, LOD was 73.0 μM and LOQ was 241.2 μM.

A typical chromatogram of a real urine sample with the addition of sarcosine (400 μM). The sample was adjusted to pH 2.1 with HCl.

Conclusions

We have developed a sensitive method for the detection of sarcosine in ultrapure water above 2 μM, in dilution buffer above 0.5 μM and in urine sample above 10 μM of sarcosine. The sample optimization method showed that the highest sarcosine sensitivity was achieved with a sample at pH of 2.1.

Acknowledgements

The work was carried out with the support of SARKO TEST 165/2016.

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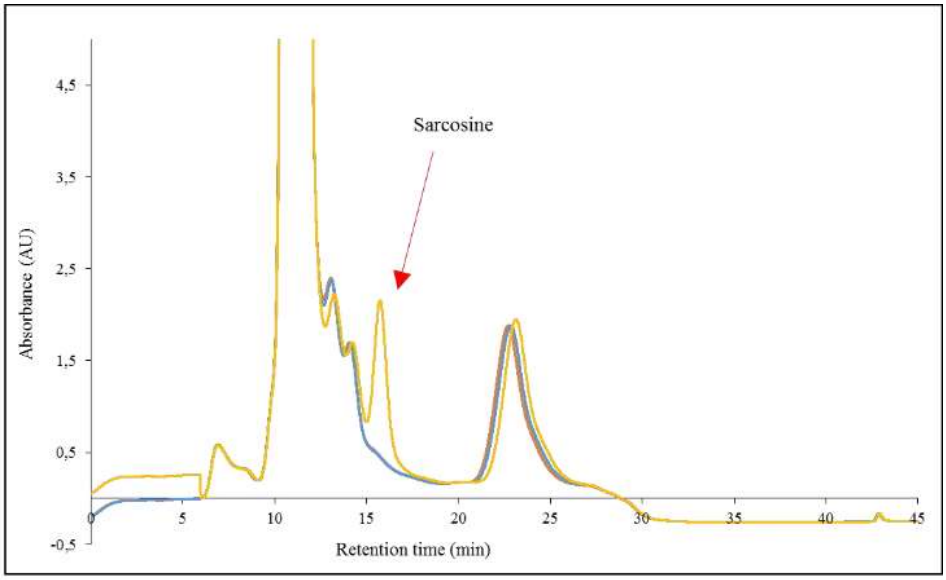

Next-generation sequencing in lung cancer diagnosis and therapeutic strategies

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Lung cancer is the leading cause of cancer-related deaths in the world, accounting for the 25% of cancer mortality. 75% of the patients are diagnosed with lung cancer at its advanced-stage, when treatment options are limited [1]. These alarming reports show the need of looking for an effective diagnostic and therapeutic strategies in early-stage lung cancer.

The last two decades have seen exponential developments of the genetic and epigenetic understanding of oncogenic transformation. Lung cancer is the end result of multistage cancerogenesis, with gradually increasing genetic and epigenetic changes, leading to oncogene activation and/or loss of the suppressor gene function [2]. Molecular analysis of these actionable mutations requires a novel technologies, that will become routine practice in lung cancer diagnosis. In this review, we took a look at the most promising platform in cancer investigetion – next-generation sequencing (NGS).

NGS is capable of sequencing millions or billions of DNA molecules simultaneously, that affords maximal tumor genomic assessment. DNA sequencing by NGS includes whole-genome, whole-exome, and targeted sequencing [3, 4]. Targeted-NGS has a potential to revolutionize clinical diagnosis of lung cancer through multiplexed detection of genomic alterations and the analysis of cancer driver genes for precision cancer therapy. Exemplary gene-panel of somatic mutations includes BRAF, EGFR, ERBB2, KRAS, NRAS, PIK3CA, PTEN and TP53, where EGFR and TP53 exhibite the highest mutation rate, and may be a therapeutic target in lung cancer patients [5,6].

NGS may detect actionable mutations with high accuracy and is a promising tool for the analysis of molecular targets for the initial diagnosis of disease, monitoring of disease progression, and identifying the mechanism of drug resistance.

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Innovative liposomes systems as transporters of active cargo

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Despite the continuous development of medicine, the application of many actives substances are limited. It is associated with their low water solubility, potential side effects and unfavorable pharmacokinetics. For this reason, the nanocarriers are very important structures that are used to create effective and at the same time safe drug delivery systems. Currently, liposomes are the most intensively studied delivery systems of active substances. It is connected with their numerous properties such as

biocompatible, non-toxicity and a very good ability to encapsulate hydrophilic and hydrophobic ingredients. Moreover, the liposomes are composed of natural components of biological membranes – phospholipids, what give them better biocompatibility and biodegradability [1].

One of the key parameter in designing nanoscopic drug delivery systems is the size of liposomes, which influence their properties and application. The smaller nanocarriers are less captured by mononuclear phagocyte system (MPS) cells. They have also improved accumulation in cancerous tissues which is connected with the occurrence of the enhanced permeability and retention (EPR) effect. Liposomes of various sizes can be obtained by utilization of different techniques. The main popular way to create multilamellar liposomes MLV (above 1 μm) is the thin-film hydration method. The received MLV dispersion is submitted to sonication or extrusion in order to obtain the smaller nanocarriers (< 200 nm) [2].

The therapeutic index of biologically active substances encapsulated in liposomes is higher in comparison to drugs transported in free form. This is due to reduction of the exposure of healthy tissues to the encapsulated drugs. Furthermore, functionalization and numerous surface modifications of vesicles permit to obtain stable liposomes which can allow transport drugs to the target tissues. It is also possible to control the release of active cargo due to the action of a specific factor (e.g. temperature, pH, ultrasound, magnetic field or laser irradiation) [3]. Different kind of modified liposomes may contribute to development of the innovative and effective treatments for many diseases (including cancer). Due to that fact there is a huge need to conduct studies in this field.

Acknowledgements

The support of statutory activity subsidy from the Polish Ministry of Science and Higher Education for the Faculty of Chemistry of Wroclaw University of Science and Technology is gratefully acknowledged.

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Modulation of blood-brain barrier permeability by activating adenosine A2 receptors in oncological treatment

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The blood-brain barrier (BBB) plays an important protective role in the central nervous system and maintains its homeostasis. It regulates transport into brain tissue as well as protects neurons against the toxic effects of endo- and exogenous substances circulating in the blood, thus providing proper functioning of the central nervous system [1].

However, in case of neurological diseases or primary brain tumors, i.e. gliomas, the higher permeability of the blood-derived substances in the brain tissue is necessary. Modulation of the blood-brain barrier

permeability may contribute to an increase in the concentration of the drug in the CNS and thus increase the effectiveness of therapy.

Currently applied methods of treatment for the primary brain neoplasms include surgical tumor removal, radiation therapy, and chemotherapy. Despite the above-mentioned treatment methods, the prognosis of primary brain tumors still remains bad (for instance, malignant glioma median survival is less than 12 months) [2]. Moreover, chemotherapy options seem to be limited due to low drug penetration into the cancerous tissue. Therefore, further research is required to increase therapeutic options in patients with brain tumors. The aim of the article is to assess the possibility to increase the BBB permeability to increase the effectiveness of oncological therapy.

In this case, the Adenosine 2A receptor ($A_{2A}R$) seems to be a promising therapy-target, due to its important role in the modulation of BBB. The action of A2A agonists increases the permeability of the blood-brain barrier by actin-cytoskeletal reorganization and acting on the tight junctions [3]. Interestingly, it has been proven that the gene encoding this receptor is overexpressed in the tumor area. Moreover, clinical trials using the chemotherapy agent (Image-guided paclitaxel injection) together with an A2A nano-agonist showed a better antitumor effect and prolonged survival [4].

Adenosine 2A receptor modulation may be a potential target to increase the effectivity of chemotherapeutics and to improve the results of cancer therapy.

Acknowledgments: The work was created as part of the activity of the Student Research Group "Biology of Cancer Cell" at the Wroclaw Medical University (SKN No. K 148) and Statutory Funds of Department of Molecular and Cellular Biology No.: SUB.D260.20.009

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The influence of disiloxane derivatives on activity and expression of ABCB1 transporter in colon cancer cells

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Background

Since chemotherapy continues to be a method of choice for the treatment of cancer, any factors that undermine its effectiveness constitute a serious therapeutic issue. In majority of patients the initial response to chemotherapy is satisfactory, however the consequent occurrence of multidrug resistance (MDR) results in a development of progressive disease. Among many mechanisms that may lead to MDR, the overexpression of ATP Binding Cassette (ABC) transporters such as ABCB1 protein

(P-glycoprotein, MDR1) seems to be the most important. ABCB1 is a transporter that utilizes energy gained from hydrolysis of ATP to pump many structurally variable substrates (including anticancer drugs) out of the cell [1]. The most basic idea to overcome MDR is to use the inhibitor of transporter (MDR modulator) along with chemotherapy in hope to increase intracellular accumulation of an anticancer drug and to improve the final outcome of the treatment.

Material and Methods

The pair of human colon cancer cell lines – sensitive and resistant to doxorubicin (LoVo and Lovo/Dx) as well as MDCK calls transfected with human ABCB1 gene have been employed as a model system to study the influence of putative MDR modulators on ABCB1 expression and transport activity. Cytotoxicity of the modulators was measured via SRB assay. Isobolographic analysis was employed to study putative synergism between doxorubicin and modulators. Accumulation of ABCB1 substrates was monitored by functional tests based on intracellular fluorescence measurement as well as by fluorescence microscopy. Protein expression level was assessed by Western blot.

Results

Two disiloxane derivatives were relatively cytotoxic both to colon cancer cells and to MDCK cells (recorded IC50 values were below 25 μM). Each compound was tested as a putative MDR modulator in concentration c.a. 10 times lower than its IC50 value. Both derivatives were demonstrated to increase the sensitivity of LoVo/Dx cells to doxorubicin. The existence of synergism between doxorubicin and the studied modulators was also demonstrated. Additionally, the increased accumulation of anticancer drug within resistant cells was demonstrated in the presence of the studied derivatives. The spatial arrangement of doxorubicin was affected by them, too. The inhibition of transport activity of ABCB1 protein by disiloxane derivatives was observed both in LoVo/Dx cells and in MDCK cells overexpressing human ABCB1 transporter. Rhodamine 123 was used as fluorescent substrate analogue. Its accumulation was significantly increased in both types of cells treated by the modulators. Moreover, both studied derivatives strongly reduced ABCB1 protein expression in doxorubicin-resistant colon cancer cells.

Discussion and conclusions

Two studied disiloxane derivatives were demonstrated to be effective MDR modulators in doxorubicin-resistant colon cancer cells. Used in in concentrations in which they were non-toxic to the cells they were able to increase sensitivity of resistant cells to anticancer drug. Two processes seemed to be responsible for MDR-reversal activity of the studied compounds. They both inhibited transport activity of ABCB1 and decreased the expression of this transporter.

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Morphological changes in the ovarian cortex in the early stages of diabetes mellitus in conditions of chronic stress

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