2nd Wroclaw Scientific Meetings

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Editors:
Julita Kulbacka
Joanna Weżgowiec
Nina Rembiałkowska

Wroclaw 2018

2nd Wroclaw Scientific Meetings

Wroclaw, 2nd March 2018

Editors:
Julita Kulbacka
Joanna Wężgowiec
Nina Rembiałkowska

Typesetting:
Monika Maciąg

Cover design:
Marcin Szklarczyk
Nina Rembiałkowska

ISBN 978-83-65932-02-0

Publisher:
Wydawnictwo Naukowe TYGIEL Sp. z o.o.
ul. Głowackiego 35/341, 20-060 Lublin
www.wydawnictwo-tygiel.pl

Scientific Committe:

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Department of Medical Biochemistry, Wroclaw Medical University, Poland

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Department of Toxicology, Wroclaw Medical University, Poland

prof. dr hab. Kazimiera Wilk
Department of Organic and Pharmaceutical Technology, Wroclaw University of Sicence and Technology, Poland

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

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Department of Biology, Vytautas Magnus University in Kaunas, Lithuania

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Department and Clinic of Thoracic Surgery, Wroclaw Medical University, Poland

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Institute of Immunology and Experimental Therapy, Poland

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Department of Medical Biochemistry, Wroclaw Medical University, Poland

dr hab. inż. Julita Kulbacka
Department of Medical Biochemistry, Wroclaw Medical University, Poland

dr inż. Anna Choromańska
Department of Medical Biochemistry, Wroclaw Medical University, Poland

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DIVE IN AI, Polish Japanese Academy of Information Technology, Poland

Dawid Przystupski
Department of Medical Biochemistry, Wroclaw Medical University, Poland

BioNanopor

PC Members:

prof. dr hab. Janina Kwiatkowska-Korczak
Department of Medical Biochemistry, Wroclaw Medical University, Poland

prof. Thomas Vernier
Frank Reidy Research Center for Bioelectrics, Old Dominion University, USA

prof. dr hab. Agnieszka Piwowar
Department of Toxicology, Wroclaw Medical University, Poland

dr hab. Jolanta Saczko, prof. nadzw.
Department of Medical Biochemistry, Wroclaw Medical University, Poland

dr hab. inż. Małgorzata Kotulska, prof. nadzw.
Department of Biomedical Engineering, Wroclaw University of Sicence and Technology, Poland

dr hab. inż. Julita Kulbacka
Department of Medical Biochemistry, Wroclaw Medical University, Poland

dr inż. Joanna Rossowska
Institute of Immunology and Experimental Therapy, Poland

dr inż. Anna Choromańska
Department of Medical Biochemistry, Wroclaw Medical University, Poland

dr inż. Izabela Pawlaczyk-Graja
Department of Organic and Pharmaceutical Technology, Wroclaw University of Sicence and Technology, Poland

dr inż. Joanna Weżgowiec
Department of Experimental Dentistry, Wroclaw Medical University, Poland

mgr Olga Michel
Department of Medical Biochemistry, Wroclaw Medical University, Poland

mgr inż. Justyna Mączyńska
Department of Medical Biochemistry, Wroclaw Medical University, Poland; The Institute of Cancer Research, UK

Orgnizers:

Sponsors:

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Dear Guests,

Let me shortly present the unusual, rousing history of Wrocław, and its scientific background.

Not many cities have been ruled by so many monarchies and states. The story of Wrocław begins with the establishment of a stronghold along the Amber Road on what now is ***Ostrów Tumski*** *by the Slavic tribe in the 8th century. However, the name of the fortress was first recorded in the 10th century under the name '****Vratislavia****'. It is thought to have been derived from the name of the Bohemian duke Vratislav I. From that time up to the year 1335 Wroclaw was ruled by princes from the Polish Piast Dynasty. Afterwards, the city and all the Lower Silesia province has been under the government of several monarchies and states in turn. During four centuries the town was ruled by the Bohemian emperors from Prague and the Habsburg dynasty. It was Tsar Leopold I who laid the foundation for academic life in Wrocław by establishing* ***a two-faculty university*** *– the Society of Jesus Academy. From the half of the 18th century until the end of the II World War Wrocław was under the Prussian and German government.*

In 1945 Wroclaw was ruined in 80%. Nevertheless, the team of scholars arrived at a still burning city in May to restore the academic and scientific life. Students were recruited, and the teaching and scientific activity commenced in November at the Wroclaw University of Technology.

Before the war, the scientific life of the city was intensive and fruitful, with its 9 Nobel Prize winners and many prestigious scientists, such as

Purkynê, Alzheimer and Neisser. After the war, a great effort was put to carry on good traditions.

As is known from written memories and testimonies of those who remember these times, the young students and graduates were working very hard, with enthusiasm and sacrifice. Times and generations have changed, but not the work ethic of many young people. This Conference is an example of scientific passion and enthusiasm to promote knowledge and exchange of experience by the young generation of scientists from Wrocław.

We would like to encourage you to have a tour around our city. There are no traces of the postwar devastation. The magnificent churches, the City Hall, the University and many beautiful parks are restored. Several Museums, the National Forum of Music, the Zoo and many other places open their doors to the curious visitors.

Prof. dr hab. Janina Kwiatkowska-Korczak

CONFERENCE PROGRAM

CONFERENCE PROGRAM

9:00 – 10:00 Registration

10:00 – 10:15 Opening ceremony – Prof. Jolanta Saczko and Prof. Andrzej Gamian

10:15 – 11:00 Lecture of Prof. Thomas Vernier (Old Dominion University, Norfolk, USA) – *Nanoscale electromanipulation of cell membranes – Perturbation, reorganization, restoration*

11:00 – 12:00 1st session of young scientists (8-10 min presentations), moderators: Prof. T. Vernier and Prof. J. Saczko

11:00-11:10 Paulina Komorek – *Conformational insights of Lysozyme adsorption onto the gold surface – an important factor in Alzheimer's disease diagnostics*

11:10-11:20 Sylwia Baluta – *The future in modern medical diagnostics – biosensors and sensors as the appliance for the point-of-care testing*

11:20-11:30 Agnieszka Uryga – *The relationship between the near-infrared regional cerebral desaturation and outcome in aneurysmal subanachroid haemorrhage*

11:30-11:40 Jakub W. Wojciechowski – *Forecasting contact prediction accuracy improves residue-residue contacts prediction*

11:40-11:50 Maciej Wilk – *Phytochemicals of Cistus incanus L. received in the multi-step isolation process*

11:50-12:00 Dawid Przystupski – *How Martian environment affects human cells exposed to variable temperature, pressure, overload and radiation in the stratosphere?*

12:00 – 12:30 Coffee break and POSTER SESSION

12:30 – 14:00 2nd session of young scientists (8-10 min presentations), moderators: Prof. Saulius Šatkauskas and dr hab. J. Kulbacka

12:30-12:40 Agnieszka Szczygieł – *Immunomodulation of anticancer response after chemotherapy with methotrexate nanoconjugates*

12:40-12:50 Agnieszka Buś – *Sirtuins as modulators of development and diagnostic factors of neurodegenerative diseases*

12:50-13:00 Monika Pichla – *Anticancer properties of biotinylated PAMAM G3 dendrimer conjugated with COX-2 inhibitor and PPARγ agonist*

13:00-13:10 Justyna Kutkowska – *Molecular Mechanism of Death of Non-Small Cell Lung Cancer Cells Treated with Combination of Sorafenib and* Betulinic Acid

13:10-13:20 Marta Czwojdzińska – *Olivacine anticancer activity to cancer cell lines*

13:20-13:30 Klaudia Braczyk – *The impact of Tamoxifen or Cisplatin with dichloroacetate (DCA) on the cytotoxicity with breast cancer cell lines T47D, MDA-MB-231 and MCF-7*

13:30-13:40 Łukasz Lewandowski – *The association of copper-zinc superoxide dismutase activity with oxidative damage in lungs, in reference to chronic obstructive pulmonary disease*

13:40-13:50 Bartosz M. Antoniszyn – *The antioxidant potential of the compounds isolated from leaves of Rubus* plicatus *(*Whe. *Et N. E.)*

13:50-14:00 Natalia Anger – *Characterization of murine MC38 colon carcinoma microenvironment after application of lentivectors silencing IL-10 or IL-10R expression*

14:00 – 15:00 Lunch and POSTER SESSION

14:00 – 15:00 Flow Cytometry workshops – SYSMEX PARTEC

15:00 – 15:45 Lecture of Prof. Kazimiera Wilk (Wroclaw University of Science and Technology, Wroclaw, Poland) – *Engineering multifunctional nanocarriers for therapy and bioimaging*

15:45 – 17:15 3rd session of young scientists (8-10 min presentations), moderators: Prof. K. Wilk and Prof. A. Piwowar

15:45-15:55 Dawid Lupa – *A facile synthesis of gold shell, polymer core raspberry-like microcomposites – a potential biosensors*

15:55-16:05 Magda Juszczyk – *PCA and MNF denoising method optimization for IR biomedical imaging*

16:05-16:15 Agnieszka Kamińska – *A unique spectral signature of urine extracellular vesicles for kidney damage screening in diabetic patients*

16:15-16:25 Wiktoria Wójcik – *Excessive fructose consumption – more adverse and toxic than an overabundance of glucose intake?*

16:25-16:35 Sebastian Wawrocki – *Serum IP-10 levels in active and latent tuberculosis*

16:35-16:45 Agnieszka Kamińska – *Uterine Smooth Muscle Tumors of Uncertain Malignant Potential (STUMP) – a case report*

16:45-16:55 Sabrina Dobroszek – *Regulation of hypoxia-induced factors in pituitary adenoma*

16:55-17:05 Kinga Woźniak – *An active component of propolis and ginger – in vitro study on human melanoma cells*

17:05-17:15 Natalia Niedzielska – *Absolute quantification of genes from* csg *operon in biofilm – forming bacteria*

17:15 – 17:30 Coffee break

17:30 – 18:15 Lecture of Prof. Saulius Šatkauskas (Vytautas Magnus University, Kaunas, Lithuania) – *DNA electrotransfer mechanisms: in vitro and in vivo studies*

18:15 Announcement of the results for the best presentation and closing ceremony, certificates, and goodbye

19:00 Wroclaw by night and downtown meeting ….

LECTURES

L 1.

Nanoscale electromanipulation of cell membranes – Perturbation, reorganization, restoration

P. Thomas Vernier

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

We are immersed in electric fields. Every sensation, every motion, every thought arises from a coordinated network of electrical signals – our nervous system. Electricity can kill us, it can restart our heart, it can control and energize our paralyzed limbs. Yet we know surprisingly little about the details of the interactions between electric fields and living cells. Most of what we have learned is centered on the cell membrane, the primary transducer of the energy of externally applied electric fields, and the mediator of intercellular signaling.

Of great interest are the effects of electric pulses that are intense enough to affect membranes but brief enough that they do not cause permanent damage. This is the regime of electropermeabilization (electroporation), where we use pulsed electric fields to modify the barrier function of the membrane to allow normally impermeant pharmacological agents or genetic material to gain access to the cell interior. Applications of electroporation technology include cancer therapy, genetic engineering, and bio-industrial processing. Our investigations of electropermeabilization on the nanoscale, with the tools of molecular modeling and quantitative fluorescence microscopy, reveals an amazing biomolecular complexity, even within a few nanometers, a few nanoseconds.

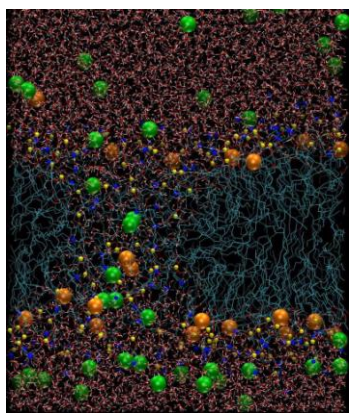

Figure 1. Calcium chloride transport through a molecular model of a lipid electropore

Prof. Thomas Vernier *is Research Professor at the Frank Reidy Research Center forBioelectrics at Old Dominion University in Norfolk, Virginia, USA. His research and industrial experience includes ultraviolet microscopy analysis of S-adenosylmethionine metabolism in a psychrophilic strain of the yeast Rhodotorulaglutinis, molecular biology of the temperature-sensitive host restriction of bacterial viruses in Pseudomonas aeruginosa, low-level environmental gas monitoring, wide-band instrumentation data recording, physical and electrical characterization and modeling of semiconductor and microelectromechanical devices, and the integration of cellular and biomolecular sensors, carbon nanotubes, and quantum dots with commercial integrated electronic circuit fabrication processes. Vernier currently studies the effects of electric fields on biological systems, with applications in cancer therapeutics, combining experimental observations with molecular dynamics simulations. His focus is on understanding the biophysical mechanisms that govern electric field-driven, nondestructive perturbations of biological membranes.*

L 2.

Engineering multifunctional nanocarriers for therapy and bioimaging

Kazimiera A. Wilk

Department of Organic and Pharmaceutical Technology, Wroclaw University of Science and Technology, Wroclaw, Poland

One of the most important tasks of surface and polymer chemistry or nanomedicine is the development of multifunctional colloidal drug delivery systems (DDS), i.e., theranostic nanoparticles, multiple drug nanovehicles, smart DDS, that can transport simultaneously many unique components, including therapeutic (cytostatics, photosensitizers, drugs of a natural origin such as e.g. flavonoids) and diagnostic (organic dyes, semiconductor nanocrystals, upconverting nanocrystals) agents within a single nanocarrier (for the general idea see Fig.1). In particular, such nanocarriers may be designed to transport hydrophobic cargo to the site of disease. Co-encapsulation of selective and active compounds into a variety of polymeric nanocarriers can be performed using different strategies, including the following: selfaggregation, interfacial deposition – co-solvent removal, interfacial polymerization or/and coreshell entrapment, physical adsorption and chemical immobilization or conjugation. Such approaches may in principle permit targeted cancer therapy, combination cancer therapy, molecular diagnosis, and − in case of nanotheranostics − simultaneous monitoring and treatment. One of the key functions of a variety of nanocarrier-based delivery systems (the threshold nanoparticle sizes <200 nm for extravasation into tumors) is to serve as shields to protect the drug molecules from premature degradation and unexpected harmful side-effects from degradation and various toxic interactions with the biological environment. Other crucial features of such nano-co-encapsulated systems are in the fields of efficacious drug loading capacity, chemical and physical stability, sustained and successful delivery to the target cells, as well as selective accumulation in the malignant tissues without damaging the healthy cells.

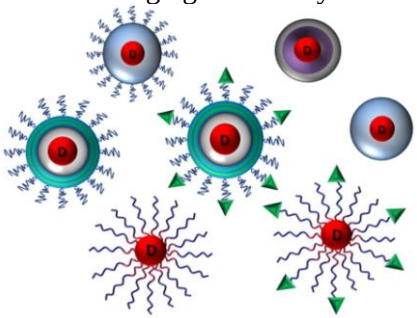

Figure 1. The schematic presentation of the custom-designed multifunctional nanocarriers

Prof. dr hab. inż. Kazimiera A. Wilk *is a full professor at the Wroclaw University of Science and Technology (WUST), Head of the Department of Organic and Pharmaceutical Technology (Faculty of Chemistry, WUST). Research profile: colloidal polymeric nano- and microcarriers of drug molecules (e.g., photosensitizers and cytostatics) and biologically active compounds (e.g., flavonoids, antioxidants of natural origin), nanotheranostic systems, co-encapsulation processes, the design of novel micellar systems (nanoemulsions, cosmetic emulsions, polymeric micelles) and cosmeceuticals.*

L 3.

DNA electrotransfer mechanisms: *in vitro* and *in vivo* studies

Saulius Šatkauskas

Vytautas Magnus University, Kaunas, Lithuania

Gene electrotransfer into cells and tissues is a complex process involving multiple steps that lead to plasmid DNA passage from the extracellular region to the cell nucleus crossing the barriers of the plasma membrane, cytoplasm and nucleus membrane. It is well documented that plasmid DNA, during application of electric pulses first forms aggregates in the electroporated membrane, which then are translocated into the cytoplasm. Electrical parameters of pulses used for gene electrotransfer affect the initial steps of DNA-membrane interactions. At later stages, translocation of the aggregates across the membrane is related with some endocytotic pathways. Several studies employing various endocytosis inhibitors has shown that that these inhibitors decreased transfection efficiency both *in vitro* and *in vivo*. Whatever the mechanism of the DNA uptake, our studies show that translocation of DNA through the membrane leads to increase in membrane permeability for small molecules. When considering gene electrotransfer into tissues it becomes clear that other nonelectrical conditions are also of primary importance.

Prof. Saulius Šatkauskas *is a professor in Department of Biology, Vytautas Magnus University in Kaunas (Lithuania). He is also head of Biophysical Research Group. His research lies in the fields of membrane electropermeabilization and sonoporation in cells and tissues.*

ORAL PRESENTATIONS

O 1.

Conformational insights of Lysozyme adsorption onto gold surface – an important factor in Alzheimer's disease diagnostics

P. Komorek, M. Wałek, B. Jachimska

Jerzy Haber Institute of Catalysis and Surface Chemistry PAS, Cracow, Poland
email: ncjachim@cyf-kr.edu.pl

Lysozyme (LYS) aggregation and amyloids are linked to evolution of neurodegenerative diseases such as Alzheimer's and Parkinson's. In this work, the adsorption of lysozyme onto a gold surface under environmentally relevant conditions (pH, time of adsorption, ionic strength) was investigated through the use of two methods: Multi-Parameter Surface Plasmon Resonance (MP-SPR) and Quartz Crystal Microbalance with Dissipation Monitoring (QCM-D). MP-SPR and QCM-D methods allow to monitor adsorption and desorption processes quantitatively. Additionally, combined these studies allow determining the degree of hydration of protein monolayers as a function of pH and ionic strength. The association (k_a), dissociation (k_d) rate constants and the affinity constant (K_D) of LYS molecules on the gold surface were determined. These parameters indicate that the LYS monolayers remain irreversible adsorbed on the gold surface. The stability of the lysozyme conformation adsorbed onto gold surface molecules was explored through the spectroscopic technique known as Polarization-Modulation Infrared Reflection-Absorption Spectroscopy (PM-IRRAS). According to the results obtained, a consequence of adsorption onto gold is the misfolding of the lysozyme when compared with its conformation in solution. Misfolding of the lysozyme in extreme pH conditions is a crucial observation because changes in its secondary structure can cause the formation of aggregate particles and amyloids. The concentration, ionic strength and time of adsorption had minimal effect on the degree of misfolding of the lysozyme. Additionally, the contact angle measurements (CA) showed that the hydrophilic nature of lysozyme adsorbed on gold surface increases with higher concentration and ionic strength of protein solution.

ACKNOWLEDGEMENTS: This work was supported by National Science Centre (NCN) OPUS 2016/23/B/ST5/02788. Paulina Komorek acknowledges the support of InterDokMed project no. POWER.03.02.00-00-I013/16.

O 2.

The future in modern medical diagnostics – biosensors and sensors as the appliance for the *point-of-care* testing

S. Baluta, J. Cabaj

Department of Chemistry, Wroclaw University of Science and Technology, Wrocław, Poland
email: sylwia.baluta@pwr.edu.pl

Rapid and sensitive neurotransmitters detection (i.e. epinephrine, dopamine, norepinephrine) is extremely important in modern medicine. These compounds mainly occur in the brain and central nervous system of mammals. Any changes in the neurotransmitters concentration may lead to many diseases, such as Parkinson's or schizophrenia. However, there is any available device, which will show concentration of these neurotranmistter in patient's body. Classical techniques of chemical analysis, despite many advantages, do not permit to obtain immediate results or automatization of measurements. Chemical sensors have displaced the conventional analytical methods – sensors combine precision, sensitivity, fast response and the possibility of continuous-monitoring.

Our research is focused on develop optical and electrochemical biosensors or sensors for neurotransmitters detection. In developed optical biosensor for detection of dopamine, we used graphene quantum dots (GQDs) for detection system. In such sensor dopamine molecules coats the GQD surface – in result occurs quenching of fluorescence due to Resonance Energy Transfer (FRET). Changes in fluorescence correspond to specific concentrations of the neurotransmitter in tested sample, so it is possible to accurately determine the concentration of dopamine in the sample. Our research also has proved facile and convenient method for epinephrine, norepinehrine and also dopamine determination based on laccase and tyrosinase-based oxidation of catecholamine derivatives. The resulting sensors (built of electrode modified with graphene quantum dots or semiconducting polymer and laccase) exhibits good performance, strong affinity between enzyme and neurotransmitter, fast response to the substrate and good linear range. Such systems could be used in medical diagnostics for neurotransmitters detection.

ACKNOWLEDGEMENTS: The authors are gratefully thanking to Wroclaw University of Science and Technology for the financial support.

O 3.

The relationship between the near-infrared regional cerebral desaturation and outcome in aneurysmal subanachroid haemorrhage

A. Uryga[1], M. Burzyńska[2], M. Kasprowicz[1]

[1]*Department of Biomedical Engineering, Faculty of Fundamental Problems of Technology, Wroclaw University of Science and Technology, Wroclaw, Poland*
[2]*Department of Anesthesiology and Intensive Care, Wroclaw Medical University, Wroclaw, Poland*
email: agnieszka.uryga@pwr.edu.pl

Non-invasive continuous monitoring of cerebral regional saturation (rSO_2) in aneurysmal subanachroid haemorrhage (aSAH) patients provides information on brain oxygen demand-supply balance and could be interpreted as early warning factor [1]. The aim of the study was to investigate the relationship between cerebral desaturation, short-term outcome and neurophysical impairment in aSAH patients.

The rSO_2 monitoring was performed in 38 patients (age 57±15 years) using near-infrared spectroscopy (NIRS) during 3.4±1.8 days in continuous mode. The rSO_2 value of <60% for at least 30 minutes was defined as cerebral desaturation. Short-term outcome was assessed with Glasgow Outcome Scale (GOS) at discharge from the hospital. The study was approved by the local bioethical committee of Wroclaw Medical University (KB-134/2014).

9 patients (24%) died during ICU stay. Neurological deficits after aSAH occurred in 17 patients (45%). 28 patients (74%) had good short-term outcome (GOS IV-V). Cerebral desaturation was found in 17 patients (45%). The median rSO_2 ipsilateral to the aneurysm was lower in patients with cerebral desaturation episodes than in subject without these episodes (69.00±4.81[%] vs. 74.00±10.79[%]; p=0.019). Patients with cerebral desaturation episodes more frequently died than those ones without (15.79% vs. 35.29%, p=0.05). Patients with rSO_2 value of <60% and poor outcome have longer duration of cerebral desaturation episodes in compare to those with good outcome (5.71±4.00 [hrs.] vs. 1.78±1.47 [hrs.]); p=0.011).

The outcome and mortality were related with cerebral desaturation episodes in aSAH patients. The early detection of cerebral desaturation during the continuous monitoring of rSO_2 may allow for the personalised therapy, leading to improve patients' outcome.

ACKNOWLEDGEMENTS: This study was funded by the National Science Centre (Poland) under Grant No. 2013/09/B/NZ4/01343. A. Uryga received a scholarship for this study, granted to the Faculty of Fundamental Problems of Technology, Wroclaw University of Science and Technology by the Minister of Science and Higher Education in 2017 for research of young scientists and PhD students.

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O 4.

Forecasting contact prediction accuracy improves residue – residue contacts prediction

J.W. Wojciechowski[1], P.P. Woźniak[2]

[1]*Faculty of Chemistry, Wrocław University of Science and Technology, Wrocław, Poland*
[2]*Department of Biomedical Engineering, Faculty of Fundamental Problems of Technology, Wroclaw University of Science and Technology, Wrocław, Poland*
email: kubwoj95@gmail.com

Experimental methods used to solve the three-dimensional protein structure are expensive and time consuming. In order to overcome this problem, many computational approaches were proposed that use amino acid sequence only. Currently, the best such methods start with the prediction of residue-residue contacts that are later used to model protein structure. Best residue-residue contacts prediction methods are based on direct coupling analysis (DCA) of correlated mutations [1]. Unfortunately, they are able to predict only 40% contacts correctly out of 100 highest scored residue pairs. In this study we propose methods that improve the performance of gplmDCA method [2], using its forecasted contact prediction accuracy combined with contact groups analysis and neural networks.

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O 5.

Phytochemicals of Cistus incanus L. received in the multi-step isolation process

M. Wilk, I. Pawlaczyk-Graja, K. A. Wilk

Department of Organic and Pharmaceutical Technology, Faculty of Chemsitry, Wrocław University of Science and Technology. Wrocław, Poland
email: maciej.wilk94@outlook.com

Cistus incanus L., also known as *Cistus creticus* or *Cistus villosus* is a typical Mediterranean shrub species distributed along the coastal belt of the Central-Eastern Mediterranean, in Southern Europe, Western Asia and Northern Africa. According to ancient ethnobotanical use of genus Cistus, simple decoctions from plant leaves are an effective remedy for several microbial disorders and infections [1]. Due to the presence of polyphenols and flavonoids, Cistus species are widely appreciated for their potential pro-health effects, i. e. antibacterial, antiinflammatory, antiviral, antiulcerogenic, cytotoxic and antifungal activities [2]. Nowadays, numerous manufacturers offer herbal infusions of Cistus incanus (Cistus tea) or dietary supplements derived from this plant or its extracts.

Water-soluble extracts were obtained in multi-step isolation process [3]. Chemical characterization of the received extracts using colorimetric analysis was done.

ACKNOWLEDGEMENTS: This work is supported by Wrocław University of Science and Technology, Wrocław, Poland. Part of analyses were made on the instruments purchased by the Project „WroVasc – Integrated Cardiovascular Center", co-financed by the European Regional Development Fund, within Innovative Economy Operational Program, 2007-2013 realized in Regional Specialist Hospital, Research and Development Center in Wrocław.

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[4] R. Gancarz, I. Pawlaczyk, L. Czerchawski, *Patent No PL 211520 B1.*

O 6.

How Martian environment affects human cells exposed to variable temperature, pressure, overload and radiation in the stratosphere?

D. Przystupski[1], B. Ziętek[2], O. Michel[1], J. Kulbacka[1]

[1]*Department of Medical Biochemistry, Wroclaw Medical University, Chalubinskiego 10, 50-368 Wroclaw, Poland*
[2]*Faculty of Mechanical Engineering, Wroclaw University of Science and Technology, Wybrzeze Wyspianskiego 27, 50-370 Wroclaw, Poland*
email: monteek17@gmail.com

The aim of the study was the verification how the external environment can affect the functioning of cells. The experiment was carried out to send a stratospheric balloon to a height of 35 km above the surface of the Earth with human gingival fibroblasts, SKOV-3 and CHO cells. In this way, the research material got into the stratosphere, where the low temperature, pressure and UV radiation levels are similar to these currently presenting on the surface of Mars. This enabled the determination of the effect of subcosmic conditions on the functioning of human cells. There was examined whether the type of freezing medium (DMSO+FBS; Bambanker®; sucrose solution) in which the cells were suspended effects on the cells' properties and whether preincubation with various antioxidants protect cell membranes from damage and disintegration in the stratosphere. The results were compared with the data obtained from laboratory-simulated low temperature effects on cells' properties *in vitro*, which revealed that cells from different tissues respond differently to subcosmic conditions. Significant differences in the results after laboratory simulation and after the balloon flight indicate that various parameters of the extra-terrestrial environment – the pressure, radiation and the overload associated with the balloon flight have a significant effect on the functioning of cells.

ACKNOWLEDGEMENTS: The study was supported by funds from the project "Budowa mini aparatury naukowo-badawczej na pokladzie balonu stratosferycznego" financed by Wroclaw University of Science and Technology, partially from Statutory Funds of the Wroclaw Medical University No.: ST.E130.16.060 (PI prof. J. Zalewski) and from Student Scientific Club of Cancer Cell Biology.

O 7.

Immunomodulation of anticancer response after chemotherapy with methotrexate nanoconjugates

A. Szczygieł, J. Mierzejewska, N. Anger, K. Węgierek, J. Rossowska, T. Goszczyński, E. Pajtasz-Piasecka

Ludwik Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wroclaw, Poland
email: agnieszka.kubica@iitd.pan.wroc.pl

Nanoconjugates of methotrexate (MTX) and hydroxyethyl starch (HES) are a new type of therapeutic compounds that are a combination of certified drugs widely used for the current medical treatment. Conjugation of MTX with HES (MTX-HES) is an innovative solution, that prolongs the half-life of MTX and reduces its side effects.

Due to our research on development of new forms of combined chemo-immunotherapy, we determined the time-dependent immunomodulatory effect on anticancer response after chemotherapy. For this purpose, mice with subcutaneously growing murine colon carcinoma tumor (MC38) received intravenously MTX or MTX-HES. On the 3rd and 10th day of therapy, mice were sacrificed and spleens and tumors were collected. Changes in percentage of tumor infiltrating lymphocytes and in activity of splenocytes were evaluated. We observed that on 3rd and 10th day of therapy, infiltration of CD4+ and CD8+ T cells into tumor tissue in MTX-HES-group was higher than in MTX-group. In both groups the percentage of Tregs in tumors drastically decreased on the 3rd day. However, only in MTX-HES-group percentage of Tregs remained at similarly low level. Nanoconiugate administration resulted in increased percentage of CD8+ T cells among splenocytes which changed in course of experiment. Moreover, regardless of the day of therapy, cytotoxic activity of splenocytes against tumor cells was higher.

All these findings demonstrate the highest immunomodulatory effect of MTX-HES on immune system at the 3rd day after compound administration. This time-point is consistent with our assumptions of planned immunotherapy.

ACKNOWLEDGEMENTS The study was funded by National Science Centre (project no. 2015/19/N/NZ6/02908).

O 8.

Sirtuins as modulators of development and diagnostic factors of neurodegenerative diseases

A. Buś[1], A. Moniczewski[2]

[1]*Department of Toxicology Collegium Medicum, Jagiellonian University, Cracow, Poland*

[2]*Department of Toxicology Collegium Medicum, Jagiellonian University, Cracow, Poland*

email: agnieszka.aga.bus@gmail.com

Sirtuins – family of proteins, functionally – NAD(+)-dependent deacetylases. First protein, Sir2 (silent information regulator 2), was obtained from Saccharomyces cerevisiae in 1979 and shortly after that other sirtuins (Sir1-7) were studied. Their role in aging process regulation was thoroughly described in numerous studies. Although recently scientists have been focusing on the different site of their action. It came to light that sirtuins level modulation occurs in many disorders – it provided a new promising pharmacological target for prevention, diagnostics and treatment of many other disorders. As stated above, these proteins regulate aging process. Thus, it was highly possible, that they also may be involved in the delaying and treatment of age-related diseases. Results of studies in this field demonstrated that proper modulation of SIRT-1 and SIRT-2 activity decreases toxicity of alphasynuclein in Parkinson's disease and amyloid beta in Alzheimer's disease. It also protects neurons by means of decreasing oxidative stress and prevent neuronal loss in Huntington's disease and multiple sclerosis. These results clearly indicated on varied roles of sirtuins in different diseases and also mechanisms of regulations were undetermined, therefore further studies had to be conducted. In this review latest reports from scientific articles published in the last few years are summarized in the aspect of their role in the development, diagnostics and treatment of neurodegenerative disorders, such as Parkinson's, Alzheimer's disease, multiple sclerosis and Huntington's disease.

O 9.

Anticancer properties of biotinylated PAMAM G3 dendrimer conjugated with COX-2 inhibitor and PPARγ agonist

M. Pichla, Ł. Uram

Department of Polymer and Biopolymer, Rzeszow University of Technology, Rzeszow, Poland
email: monika.pichla@outlook.com

The use of targeted drug carriers might be a key to a development of highly effective anticancer treatment and it may reduce the toxicity of drugs to healthy cells. The purpose of this study was to determine the effect of the PAMAM G3 dendrimer conjugated with the 16 Celecoxib molecules, which played a role of cyclooxygenase-2 inhibitor, 15 Fmoc-L-Leucine molecules acting as a PPARγ agonist and biotin, which was a targeting molecule, on cell death type, motility, proliferation and intracellular ATP levels of squamous cell carcinoma (SCC-15) and glioblastoma (U-118 MG). Both drugs seem to exhibit a synergistic effect and improve the effectiveness of therapy by regulating specific biochemical pathways within cells. Promising results were obtained due to the toxicity of the construct at low concentrations – at 1-2 μM concentrations cells from both cell lines died mainly *via* apoptosis but at 4 μM *via* necrosis. Caspase 3 and 7 activities were significantly more visible in SCC-15 cell line. Signs of necrosis were observed only at 4 μM concentration. Biotinylated PAMAM G3 conjugated with drugs resulted with an increased level of intracellular ATP in glioblastoma cells, showed an antiproliferative activity and metastasis-relevant ability to inhibit cell migration in both cell lines. It is essential that the antiproliferative effect of the conjugate was more apparent in SCC-15 lines, while the inhibition of migration was more effective in glioblastoma. Further *in vitro* and *in vivo* studies on biotinylated compounds is necessary to optimize their therapeutic properties.

O 10.

Molecular mechanism of death of non-small cell lung cancer cells treated with combination of sorafenib and betulinic acid

J. Kutkowska, L. Strzadala, A. Rapak

Department of Experimental Oncology, Ludwik Hirszfeld Institute of Immunology and Experimental Therapy Polish Academy of Science, Wroclaw, Poland
email: justyna.kutkowska@iitd.pan.wroc.pl

Sorafenib is a multi-targeted kinase inhibitor that has shown efficacy against a wide variety of tumors including human nonsmall cell lung cancer (NSCLC). Previously reported in vitro and in vivo studies have demonstrated that betulinic acid has antitumor and anti-proliferative properties and induce apoptosis in tumor cells. Combination of drugs with different targets is a logical approach to overcome multilevel cross-stimulation among key signaling pathways in NSCLC progression. NSCLC cell lines: A549, H358 and A427, with different KRAS mutations, and normal human peripheral blood lymphocytes cells (PBL), were treated with sorafenib and betulinic acid alone and in combination. We examined the effect of different combined treatments on viability (MTS test), proliferation and apoptotic susceptibility analyzed by flow cytometry, alterations in signaling pathways by Western blotting and colony-forming ability. The combination of sorafenib with betulinic acid had a strong effect on the induction of apoptosis of NSCLC cell lines. Also, this combination was not toxic for PBL cells. Combination treatment changed the expression of proteins involved in the mitochondrial apoptosis pathway and induced apoptotic death by caspase activation. Also, the combination treatment inhibited expression or phosphorylation of ERK1/2, AKT and mTOR in NSCLC cell lines. Importantly, combination treatment with low drug concentrations tremendously reduced colony-forming ability of A549, H358 and A427 cells, as compared to both compounds alone. Our study suggests that combination therapy with low concentrations of sorafenib and betulinic acid had the capacity to induce high levels of cell death and abolish clonogenic activity in some NSCLC cell lines.

O 11.

Olivacine anticancer activity to cancer cell lines

M. Czwojdzińska, T. Gębarowski

Department of Basic Medical Sciences, Faculty of Pharmacy, Wrocław Medical University, Wrocław, Poland
email: tomasz.gebarowski@umed.wroc.pl

Introduction: Ellipticine, an alkaloid first extracted from the leaves of *Ochrosia elliptica*, is known of its antitumor effects, including interactions with DNA, topoisomerase II inhibition and impact on cell-cycle. Olivacine, which is ellipticine's natural isomer, was isolated in 1958 and is known mostly for its antimalarial effects, but its antitumor activity is still unknown.
Aim: To determine olivacine's antineoplastic properties and compare them with those showed by ellipticine.
Material and methods: Lung carcinoma cells (A549) were cultured in an adequate medium (RMPI supplemented with 10% FBS) and later seeded into 96-well plates (2,500 cells/well) and incubated for 24h. Next, cells were treated with olivacine and ellipticine at concentrations of 1, 2, 5, 10, 20 μM and incubated for 48h. Cells proliferation and cytotoxic assays were performed using SRB test. To determine natural fluorescence, cells were observed under fluorescence microscope. Collected data was analyzed by using Microsoft Excel.
Results: Olivacine showed antitumor effect on A549 cells. The effect was dosedependent and occurred at concentration as low as 1 μM (51,2% growth inhibition). 20 μM and 10 μM concentrations were most cytotoxic effect, reaching 69,6% and 66,7%, respectively.
Conclusions: In conducted studies olivacine had shown antineoplastic properties and green fluorescence similar to ellipticine.

O 12.

The impact of Tamoxifen or Cisplatin with dichloroacetate (DCA) on the cytotoxicity with breast cancer cell lines T47D, MDA-MB-231 and MCF-7

K. Braczyk[1], L. Biegańska[2]

[1]*Department of Cell Biology, Medical University of Silesia, Katowice, Poland*
[2]*Department of Cell Biology, Medical University of Silesia, Katowice, Poland*
email: lidia.m.bieganska@gmail.com

The mechanism of dichloroacetate (DCA) is based on pyruvate dehydrogenase kinase (PDK) enzyme inhibition. PDK is involved in cellular respiration where it catalyses transfer of a phosphate group from the phosphoenolpryuvate to the ADP, in result of it ATP and pyruvate are made. PDK inhibition causes activation of pyruvate dehydrogenase (PDH), increase of pyruvate concentration in mitochondria and sensitize cancer cells to apoptosis induction. The mechanism of DCA suggests the possibility of using this substance in cancer diseases such as breast cancer, caused by cellular respiration disorders. In recent years, there have appeared many research results describing application of another anticancer substances in combination therapy with dichloroacetate in order to increase effectiveness of apoptosis induction in cancer cells. The DCA alone "unlocks" cancer cells from apoptosis-resistant state that makes it an attractive pre-chemotherapy drug or may be applied simultaneously with chemotherapy or radiotherapy increasing effectiveness of other anticancer drugs, decreasing their required dosages and reducing toxicity of standard anticancer therapies.
The aim of our studies was to evaluate the impact of Tamoxifen or Cisplatin with dichloroacetate on increase of cytotoxicity with breast cancer cell lines T47D, MDA-MB-231 and MCF-7.
The studies showed a significant impact of DCA on cancer cells' viability in combination therapy with Tamoxifen or Cisplatin.

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O 13.

The association of copper-zinc superoxide dismutase activity with oxidative damage in lungs, in reference to chronic obstructive pulmonary disease

Ł. Lewandowski, M. Kepinska, H. Milnerowicz

Department of Biomedical and Environmental Analyses, Faculty of Pharmacy with Division of Laboratory Diagnostics, Wrocław Medical University, Borowska 211, 50-556 Wrocław, Poland
email: shoo12370@gmail.com

Chronic obstructive pulmonary disease (COPD) is a term, which describes progressive lung diseases, such as emphysema, chronic bronchitis, or refractory, non-reversible asthma. The inflammatory injury found in COPD is connected with oxidative stress, mainly due to long-term exposure to cigarette smoke. Oxidative stress in COPD also stems from changes in the activity of main enzymatic antioxidants in the body, such as the copper-zinc superoxide dismutase (Cu,Zn-SOD). The aim of this poster is to show alterations in Cu,Zn-SOD activity in various specimen of individuals with COPD.

Cu,Zn-SOD, mainly in its extracellular form (SOD3) contributes greatly to the total antioxidative capacity in the lungs. Regulation of SOD3 activity is one of many antioxidative mechanisms found in COPD. Being released from inflamematory cells during lung inflammation, it prevents from ceramide-induced alveolar enlargement and oxidative fragmentation of extracellular matrix, leading to emphysema. Smokers and patients with COPD exhibit an increased amount of SOD3 in sputum. Research into the activity of SOD in COPD shows: decreased total SOD activity in the saliva and bronchoalveolar lavage, decreased Cu,Zn-SOD activity in both serum and plasma. However, SOD activity is increased in erythrocytes in patients with COPD exacerbation; in patients with stable COPD, this increase is insignificant. Research suggests that single nucleotide polymorphisms (SNPs) in the *SOD3* gene, mainly rs1799895, may affect susceptibility to COPD.

SOD3 activity may be used as a marker for susceptibility to COPD exacerbation and its further implications. Future research into *SOD3* SNPs may help find association of variable lung functions and Cu,Zn-SOD activity.

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O 14.

The antioxidant potential of the compounds isolated from leaves of *Rubus plicatus* (Whe. Et N. E.)

B. M. Antoniszyn, I. Pawlaczyk-Graja, K. A. Wilk

Department of Organic and Pharmaceutical Technology, Faculty of Chemistry, Wrocław University of Science and Technology, Wrocław, Poland
email: bartosz.antoniszyn@pwr.edu.pl and izabela.pawlaczyk@pwr.edu.pl

Rubus plicatus Whe. et N. E. (*Rosaceae*) also known as the common blackberry, is an annual shrub plant, which grows almost in all around the world in numerous cultivated varieties for food, because of their sour, squashy, black fruits gathered in clusters [1]. Leaves and fruits of *R. plicatus* are also used by folk medicine, because of presence of diverse macromolecules, such as tannins and flavonoids. Extracts from blackberry are commonly used for reliving symptoms of diarrhea, because of presence of astringent agents [1]. They have also antibacterial, antifungal, antioxidant and antiinflammatory properties [2]. Using multi-step isolation [3] with some modifications the different groups of phytochemicals of leaves of *Rubus plicatus* (Whe. Et N. E.) were isolated.
The chemical character of the received fractions using different colorimetric methods was identified. The antioxidant potential of the collected *R. plicatus* extracts using ABTS, DPPH and CUPRAC assays was assessed.

ACKNOWLEDGEMENTS: This work is supported by Wrocław University of Science and Technology, Wrocław. Poland. Part of analyse was made on the instruments purchased by the Project "WroVasc – Integrated Cardiovascular Center", co-financed by the European Regional Development Fund, within Innovative Economy Operational Program, 2007-2013 realized in Regional Specialist Hospital, Research and Development Center in Wrocław.

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O 15.

Characterization of murine MC38 colon carcinoma microenvironment after application of lentivectors silencing IL-10 or IL-10R expression

N. Anger, M. Napierała, A. Szczygieł, J. Mierzejewska, K. Węgierek, E. Pajtasz-Piasecka, J. Rossowska

L. Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wrocław, Poland
email: natalia.anger@iitd.pan.wroc.pl

Tumor microenvironment (TME) is composed of immunosuppressive cells and cytokines, which inhibit the antitumor immune response and, in consequence, promote tumor growth. One of the cytokine, which increased production correlates with tumor progression, is interleukin 10 (IL-10). However, some reports indicate that IL-10 is associated with tumor growth inhibition. The aim of our research was to characterize murine MC38 colon carcinoma microenvironment after silencing of IL-10 or IL-10R expression.
Mice with subcutaneously growing MC38 tumors were intratumorally inoculated with lentivectors silencing IL-10 or IL-10R expression (shIL-10 LVs or shIL-10R LVs). After application of LVs, percentage of tumor infiltrating subpopulations of myeloid and lymphoid cells, as well as their activation stage was evaluated by multiparameter flow cytometry analysis. Moreover, suppressive activity of myeloid cells isolated from tumors was assessed in the T cell proliferation assay.
Analysis of tumors revealed that intratumorally-applied LVs transduced TME-infiltrating cells, and myeloid cells were the best recipients of the vectors. Inoculation of shIL-10 LVs caused decrease of dendritic cell percentage accompanied by reduced expression of costimulatory molecules and increase of PMN-MDSC number in TME. This effect was not observed in the group, that received shIL-10R LVs. Moreover, myeloid cells isolated from shIL-10 LV-inoculated tumors showed elevated suppressor activity towards T cells in comparison to control and shIL-10R LV-treated mice.
In conclusion, gathered data indicate that local silencing of IL-10 promote PMN-MDSC dependent suppression and did not reduce the suppressive nature of TME. The results will allow a better understanding of IL-10 role in modulation of TME and its involvement in tumor progression.
ACKNOWLEDGEMENTS: This work was financed by National Science Centre, Poland (grant no 2014/15/N/NZ4/04817).

O 16.

A facile synthesis of gold shell, polymer core raspberry-like microcomposites – a potential biosensors

D. Lupa, M. Oćwieja, Z. Adamczyk

Jerzy Haber Institute of Catalysis and Surface Chemistry, Polish Academy of Sciences, Krakow, Poland
email: nclupa@cyfronet.pl

Designing of novel, colloidal core-shell materials with controlled properties is one of the major challenges of modern nanotechnology. Among the variety of microcomposites which can be obtained using various synthetic approaches, microcomposites composed of polymeric core and gold nanoparticle (AuNP) shell attract a special attention because of their biocompatibility and unique physicochemical properties [1,2]. Therefore, the main goal of this work was to develop a simple procedure for synthesis of AuNP coated polystyrene microspheres (PSM) with controlled amount of gold shell, electrokinetic and acid-base properties. In the first stage of our work, basic physicochemical properties such as hydrodynamic diameter and zeta potential of AuNP and PSM were determined in broad range of pH and ionic strength values. Then, the AuNP@PSM microcomposites were obtained in diffusion-controlled self-assembly process. The influence of AuNP concentration and ionic strength on electrokinetic properties and coverage of AuNP monolayer was investigated. The coverage of gold shell was determined using three independent methods: concentration depletion method, direct microscopic imaging and electrokinetic method. Good compatibility of applied methods was shown, especially for low AuNP coverage. This implies that electrokinetic method can be applied directly for determination of microparticle coverage by smaller particles. Additionally, the acid-base properties of AuNP@PSM composites were evaluated. It was revealed that AuNPs immobilized at microparticles surface are much more pH-resistant than unbounded, free AuNPs in native suspension.

ACKNOWLEDGEMENTS: The authors gratefully acknowledge the financial support of the National Science Centre (OPUS Project UMO-2015/19/B/ST5/00847).

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O 17.

PCA and MNF denoising method optimization for IR biomedical imaging

M. Juszczyk[1,2], P. Koziol[1], J. Skibinska[1,3], C. Paluszkiewicz[1], W. M. Kwiatek[1], T. P. Wrobel[1], S. Urbaniak-Wasik[4]

[1]*Institute of Nuclear Physics Polish Academy of Sciences, Krakow, Poland*
[2]*Faculty of Physics and Applied Computer Science, AGH University of Science and Technology, Krakow, Poland*
[3]*Faculty of Electrical Engineering, Automatics, Computer Science and Biomedical Engineering, AGH University of Science and Technology, Krakow, Poland*
[4]*Pathology Department, Kielce, Poland*
email: tomaszpwrobel@gmail.com, tomasz.wrobel@ifj.edu.pl

Introduction: Spectroscopic imaging enables to get clinically relevant information using a chemical contrast in a non-destructive and label-free manner. The use of High Definition IR imaging in transmission mode allows to obtain the best quality spectral and spatial information about biochemical composition in the sample. However, in order to perform advanced multivariate analysis of the spectra a high Signal to Noise Ratio (SNR) is necessary. Unfortunately, it implicates extended measurement time but can be remedied by use of proper denoising approaches. The aim of this work is to present the comparison of two denoising method of PCA and MNF based on FTIR spectra of a tissue specimen and simulated data.

Methods: Principal Component Analysis (PCA) and Minimum Noise Fraction (MNF) denoising algorithms were implemented in Matlab software. In both cases, the implementation has been adopted following to the mathematical model available in the literature [1, 2]. To estimate the denoising efficiency of a simulated dataset SNR and Signal Distortion (SD) were calculated. For experimental data only, SNR was obtained.

Result: The main goal of this project was to employ spectral denoising methods to High Definition Infrared imaging results. Simulated dataset allowed to determine the optimal parameters of the methods balancing out SNR increase and loss of signal quality (SD). These parameters were later used for optimal denoising of experimental data of pancreatic cancer biopsy. The results presented here highlight the benefits and the importance of proper denoising for speeding up experimental acquisition.

ACKNOWLEDGEMENTS: This work is a part of the "Pancreatic cancer comprehensive histopathology based on IR" project, which is carried out within the Homing programme of the Foundation for Polish Science co-financed by the European Union under the European Regional Development Fund. It was performed using equipment purchased in the frame of a project co-funded by the Małopolska Regional Operational Programme Measure 5.1 Krakow Metropolitan Area as an important hub of the European Research Area for 2007-2013, project No. MRPO.05.01.00-12-013/15.

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O 18.

A unique spectral signature of urine extracellular vesicles for kidney damage screening in diabetic patients

A. Kamińska[1], M. Roman[2], K. Dziedzic-Kocurek[1], C. Paluszkiewicz[2], E. Stępień[1]

[1]*Department of Medical Physics, Marian Smoluchowski Institutute of Physics, Kraków, Poland*
[2]*Institute of Nuclear Physics, Polish Academy of Sciences, Kraków, Poland*
email: agnieszka1.kaminska@uj.edu.pl

Urine extracellular vesicles (uEVs) are membranous spherical structures released by cells lining the urinary tract. Due to the fact that these vesicles carry different biomolecules, they are considered as valuable biomarkers of kidney diseases. Diabetes is associated with numerous complications including kidney damage (nephropathy). Patients with type 2 diabetes mellitus (n=45) and healthy subjects (n=6) were enrolled to this study. Diabetic patients were divided into properly controlled (CD) and poorly controlled diabetes (UD). The discrimination criterion for diabetic patients was glycated hemoglobin level. The uEVs were isolated by hydrostatic filtration dialysis method followed by ultracentrifugation.

Raman and Infrared Spectroscopy were applied for molecular characterization of uEVs. The number and size of uEVs were measured by Tunable Resistive Pulse Sensing technology. The quality of the isolated uEVs samples was confirmed by transmission electron microscopy.

The average Raman spectra of each study group showed distinct differences in the fingerprint region. The much higher relative intensity of the Amide I band in the control compared to diabetic samples was observed. Raman spectra of the CD sample showed an additional band with a maximum at 1447 cm^{-1}, while Raman spectra of the UD showed higher relative intensity of the 1731 cm^{-1} band. Infrared spectra showed differences between control and diabetic samples in the relative intensities for 1100, 1052 and 996 cm^{1} bands which may originate from nucleic acids or carbohydrates.

A spectral signature of uEVs may serve as noninvasive diagnostic tool for kidney damage screening and discrimination of healthy subjects and diabetic patients.

ACKNOWLEDGEMENTS: The research was partially performed at the Institute of Nuclear Physics Polish Academy of Sciences using equipment purchased in the frame of the project co-funded by the Małopolska Regional Operational Programme Measure 5.1 Krakow Metropolitan Area as an important hub of the European Research Area for 2007- 2013, project No. MRPO.05.01.00-12-013/15. The study was also supported by the Polish National Centre (NCN) the grant OPUS 4 for Ewa Stępień (2012/07B/NZ5/02510).

O 19.

Excessive fructose consumption – more adverse and toxic than over abundance of glucose intake?

Wiktoria Wójcik[1], Anna Rorbach-Dolata[1], Agnieszka Piwowar[1]

[1] *Scientific group by the Department and Department of Toxicology, Faculty of Pharmacy with the Department of Medical Analytics, Wroclaw Medical University, Borowska 211, 50-556 Wrocław, Poland*
[2] *Department and Department of Toxicology, Faculty of Pharmacy with the Department of Medical Analytics, Wroclaw Medical University, Borowska 211, 50-556 Wrocław, Poland*
email: wiktoriabwojcik@gmail.com

Over the last three decades, the amount of sweeteners added to food products in developed countries has significantly increased. It is associated with a simultaneous increase both in glucose as well as fructose consumption. The first relate to the excessive consumption of fructose and the possible negative impact of a diet rich in this sugar appeared 10 years ago and since then, numerous clinical trials have been carried out, still providing important data to support this influence on an organism. Studies on the effects of fructose intake in both humans and animals have already been comprehensively analyzed and there is a strong evidence that diets with high fructose content trigger a number of adverse effects in the body, such as increased de novo lipogenesis, dyslipidemia, insulin resistance or obesity. Excessive consumption of fructose is also an important factor in the development of metabolic diseases such as non-alcoholic fatty liver disease (NAFLD) or steatohepatitis (NASH). It has also been confirmed the connection between the increased fructose consumption and the increase in uric acid concentration as a potential risk factor for the development of the metabolic syndrome. Recent studies show that the consumption of a large amount of fructose has a significant impact on increasing the number of Enterobacteriaceae, which results in adverse changes in the composition of the intestinal microflora. Moreover, it is supposed that may enhance glucose toxicity. These results suggest a existence of connection between high intake of fructose and a many metabolic changes leading to numerous disorders, although further research is still needed to fully understand the impact of excessive fructose consumption.

O 20.

Serum IP-10 levels in active and latent tuberculosis

S. Wawrocki[1], G. Kielnierowski[2], M. Druszczyńska[1]

[1]*Division of Cellular Immunology, Department of Immunology and Infectious Biology, Faculty of Biology and Environmental Protection, University of Lodz, Banacha 12/16, 90-237 Lodz, Poland*
[2]*Regional Center Hospital for Tuberculosis, Lung Diseases and Rehabilitation in Tuszyn, Szpitalna 5, 95-080 Tuszyn*
email: sebastian.wawrocki@gmail.com

Introduction: WHO estimates that over 1/3 of the living population is infected with *Mycobacterium tuberculosis* (*M.tb*). Most of the infections are latent but even among them approximately 10% can progress into active tuberculosis (TB). The development of active TB is based on immune-driven mechanisms involving cytokines and chemokines such as C-X-C motif chemokine 10 (CXCL10) known also as interferon-γ induced protein 10 (IP-10).
Aim: The aim of the study was the evaluation of IP-10 protein in serum from patients with pulmonary TB and healthy volunteers with or without latent *M.tb* infection.
Materials and methods: The study group comprised of 269 adult Polish volunteers including TB patients hospitalized at the Regional Center Hospital for Tuberculosis, Lung Diseases and Rehabilitation in Tuszyn (Poland) and healthy volunteers, who had never suffered from TB. The latent *M.tb* infection was estimated on the basis of the result of the interferon-gamma released assay (IGRA). IP-10 concentration was determined immunoenzymatically using DuoSet®ELISA Development Kit (R&D).
Results: The study showed the statistically significant increase in the levels of the chemokine in the sera from TB patients compared to the concentrations of IP-10 in IGRA(-) and IGRA(+) subjects.
Conclusion: The results showed the potential use of IP-10 as an auxiliary marker in active, but not latent tuberculosis.
ACKNOWLEDGEMENTS: This work was supported by the National Science Centre grants no 2016/21/B/NZ7/01771 and 2015/19/N/NZ6/01385.

O 21.

Uterine Smooth Muscle Tumors of Uncertain Malignant Potential (STUMP) – a case report

A. Kamińska, J. Haciuk, P. Kłębek, A. Semczuk

IInd Department of Gynecology, Lublin Medical University, Lublin, Poland
email: agnieszkamins@gmail.com

Introduction: Uterine Smooth Muscle Tumors of Uncertain Malignant Potential (STUMP) represent a group of very rare tumors which cannot be classified undeniably as benign or malignant. STUMP may be incorrectly recognized as leiomyosarcoma due to its pathomorphological image which is not simple to interpret. It is usually seen in women in their mid-forties. Typical set of symptoms involves meno-metrorrhagia, pelvic pain and pelvic mass.
Case report: A 47-year-old woman (gravida V, para III) was admitted in July 2016 to the IInd Department of Gynecology, Lublin Medical University, Lublin, Poland, due to the hypermenorrhoea and left side abdominal pain. She suffers from hereditary thrombophilia (protein C deficiency) and arterial hypertension. Gynecologic examination revealed enlarged uterus. Furthermore, an ultrasonographic scan showed a posterior wall myoma (38 mm x 35 mm) and an endometrial thickness of 12 mm. The patient was qualified to a total vaginal hysterectomy (TVH) and discharged 4 days after surgery in a good condition. STUMP was diagnosed by pathological examination. Moreover, a panel of immunohistochemical markers was performed. The tumor occurred to be SMA positive, desmin positive and CD10 negative with MIB-1 immunoreactivity of 6.6%.
Conclusions: STUMP is accidentally revealed by pathological examination leading to the fact that it does not have specific treatment plans despite excision and follow-up. Complete resection and low MIB-1 expression, which correlates with low proliferative activity, seem to be a good prognostic utility factor.

O 22.

Regulation of hypoxia induced factors in pituitary adenomas

S. Dobroszek[1], B. Rak[2], P. Wlodarski[3]

[1]*Department of Histology and Embryology, Medical University of Warsaw, Warsaw, Poland*
[2]*Department of Internal Diseases and Endocrinology, Public Central Teaching Hospital Medical University of Warsaw, Warsaw, Poland*
[3]*Department of Histology and Embryology, Medical University of Warsaw, Warsaw, Poland*
Email: sabrina.dobroszek@gmail.com

Introduction: Pituitary adenomas (PAs) are the tumors of the adenohypophysis – a central regulator of hormonal homeostasis [1]. They account for up to 15% of all intracranial tumors, and are predominantly benign [2]. PAs can be divided according to size (microadenomas and macroadenomas), and presence of hormonal activity [3]. PAs are poorly vascularized creating hypoxic conditions, which promotes proangiogenesis signalling pathways [4]. Angiogenesis is a sign of tumor progression [5]. Characterizing these pathways allows identification of invasiveness markers and anti-angiogenic therapeutic targets [6]. No reliable marker has been identified to accurately predict invasiveness and recurrence [7]. Hypoxia inducible factors HIF1α and HIF2α are transcription factors which trigger pro-angiogenic VEGF expression [8]. TGFβ is a growth factor that promotes EMT by enabling angiogenesis [9].
Objective: This experiment aimed to investigate and quantify the expression of selected genes upregulated under a gradient of hypoxic conditions.
Methods: The model pituitary adenoma cell line GH-3 was cultured in a hypoxic chamber at 3 experimental and normoxic O_2 concentrations over 96 hours. Gene expression was quantified for each concentration using real time PCR methodology at 4-time intervals. Each experiment was repeated three times, in triplicates.
Results: Some genes were upregulated over time, and expression increased in hypoxia, while others were not dysregulated.
Conclusion: Varying hypoxic conditions induces specific genes. Our results show that hypoxia related pathways may be important for pituitary adenomas development. Characterizing the behavior of genes involved in tumorigenesis provides potential for further investigation of interacting and regulating molecules, which in turn gives rise to potential therapeutic targets and tumor staging markers.

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O 23.

An active component of propolis and ginger – *in vitro* study on human melanoma cells

P. Rozborska[1], K. Woźniak[1], A. Choromańska[2]

[1]*Faculty of Chemistry, Wroclaw University of Science and Technology, Wroclaw, Poland;*

[2]*Department of Medicinal Biochemistry, Wroclaw Medical University, Poland*
Email: rozborska.paulina@gmail.com and anna.choromanska@umed.wroc.pl

Background: Melanoma is the most serious of skin tumor because it may grow rapidly and metastasize [1]. Melanoma cells have overexpression of many proteins that determine multidrug resistance, eg. Glutathione S-Transferase (GST) and glucose transporter GLUT1. That cells are characterized by high resistance to standard chemotherapy. Current forms of melanoma treatment are not very effective and have many side effects, therefore more effective therapeutic procedure are searched [2]. Caffeic acid phenetyl ester (CAPE) is a natural compound knows as the major components of propolis. It possesses antioxidant, anti-inflammatory, antibacterial, anti-viral, and cytostatic properties and has a documented toxic effect on melanoma cells [3, 4]. 6-gingerol is the most abundant bioactive compound in ginger that shows various pharmacological effects including antioxidant, antipyretic, analgesic and anti-inflammatory properties. Moreover it has anticancer activities without toxicity to normal cells [5, 6].
Objectives: The aim of this study was to evaluate in vitro the anticancer activity of 6-gingerol and CAPE on the human melanoma cell line.Materials and methods: The human malignant melanoma line MeWo was used for the experiments. The cells were grown as a monolayer in Dulbecco modified Eagle medium (DMEM, Sigma-Aldrich, USA). The viability of cells was determined by MTT assay after 24, 48 and 72 h incubation with different concentrations of CAPE and 6-gingerol (5, 10, 25, 50, 75, 100 μM).
Results: Both tested compounds reduced the viability of MeWo cells in concentration 25 μM. Our studies show that the natural compounds could become effective anticancer agents, they are promising and can be the basis for next preclinical studies.

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O 24.

Absolute quantification of genes from csg operon in biofilm – forming bacteria

N. Niedzielska[1], A. Błaszczyńska[2], M. Burdukiewicz[3], S. Rödiger[4], P. Schierack[4]

[1] Department of Biomedical Engineering, Wrocław University of Science and Technology, Wybrzeże Stanisława Wyspiańskiego 27, 50-370 Wrocław, Poland
[2]Department of Chemistry, Wrocław University of Science and Technology, Wybrzeże Stanisława Wyspiańskiego 27,
50-370 Wrocław, Poland
[3] Department of Biotechnology, University of Wrocław, Fryderyka Joliot-Curie 14a, 50-383 Wrocław, Poland
[4] Institute of Biotechnology, Brandenburg University of Technology Cottbus-Senftenberg, Universitätsplatz 1, 01968 Senftenberg, Germany
e-mail: natalaniedz@gmail.com

Objective: To investigate the correlation of strength of enteroaggregative *E. coli* (EAEC *E. coli*) biofilm formation with number of csgA gene copies and mRNA expression.
Material and methods: Four strains of *E.coli* bacteria were tested, all of which have different tendency to form biofilms [1]. These types are EAEC: 5216, 5643, 5280 and 6101 as a positive control. We used a new screening method to examine the *in vitro* biofilm formation ability, which is bound with the high prevalence of the biofilm-associated gens (operon csg). In summary, we used polymerase chain reaction (PCR) and real-time polymerase chain reaction (qPCR) to detect the biofilm-associated genes. We also made DNA sequencing.
Results: The presence of genes was evaluated by PCR and qPCR for different reference gens and primers, which had previously been published in studies [2] and verified using BLAST. All results were analyzed by R program. In this study, we can indentify association of specific genes in bacteria with increased biofilm formation.
Conclusion: The gene expression of genes from csg operon in biofilm is stronger when the biofilm formation increases in a specific *E. coli* pathotype.

ACKNOWLEDGEMENTS:
The research was carried out in the Brandenburg University of Technology Cottbus- Senftenberg in the Biotechnology Institute. This work was supported by the polish researcher Małgorzata Kotulska.

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POSTER PRESENTATIONS

P 1.

The role of cell-cell communication in physiology and pathology

W. Bartosik[1*], J. Kulbacka[2]

[1]Faculty of Biotechnology, University of Wroclaw, Wroclaw, Poland; [2]Department of Medical Biochemistry, Wroclaw Medical University, Wroclaw, Poland
email: weronikabartosik@gmail.com

A human being consists of 3.72x1013 cells. Cells require sharing information among themselves to maintain their homeostasis in the organism. Cells communicate by chemical signals, molecules generated by a sending cell to target cells, which can be detected. They are able to do this in few different ways: by direct contact, paracrine signaling, autocrine signaling and endocrine signaling.

Aberrations in cell-cell signaling are widely known as an "initiation" factor of carcinogenesis. When cells lose "contact inhibition", it causes the loss of control of proliferation and ability to respond to extracellular signals that trigger apoptosis. As long as cells are communicating each other, proliferation is not observed. Cells inhibited by chemicals (e.g., hormones, cytokines, growth factor) pose tumor promotion but this process is reversible.

This study presents the current state of knowledge of the mechanisms and analysis of the cell-cell communication. The progress in the engineering of artificial communication between mammalian cells can be observed in recent years. One of those methods is dependent on natural ligands, like nitric oxide in human lung carcinoma cells, which is secreted from the cell membrane. Another method using L-arginine has an ability to implement a communication system that is activated at large cell densities. The other application is based on sensor components in Escherichia Coli, which can produce an invasion protein that can be a cytotoxic agent for cancer. Studies on synthetic biological systems are a new discipline and the further research in this field is needed.

ACKNOWLEDGEMENTS: The research was supported by Scientific Group of Cancer Cell Biology (WMU).

P 2.

Anticancer activity of biochanin A combined with electroporation in human colorectal adenocarcinoma cells LoVo/DX

A. Błaszczyńska[1], J. Kulbacka[2]

[1]*Department of Chemistry, Biotechnological Processes, Wroclaw University of Science and Technology*
[2]*Department of Medical Biochemistry, Wroclaw Medical University, Chalubinskiego 10, 50-368 Wroclaw*
e-mail: blaszczynska.agata@gmail.com and julita.kulbacka@umed.wroc.pl

Objective: The evaluation of biochanin A effectiveness alone and in combination with electroporation (EP) against colorectal adenocarcinoma (LoVo/DX) cells with multidrug resistance in *in vitro* studies.
Materials and methods: The tests were carried out at the Biochemical Laboratory of the Wroclaw Medical University. The following electroporation parameters were applied: 8 impulses of 100 μs with the appropriate voltage for a given sample (400-2000 V/cm) at a frequency of 1 Hz each. The next stage of the experiment was to determine the cytotoxic effect of biochanin A. For this purpose, solutions with biochanin A concentrations of 0.0625-1 μmol/ml were used. In the last stage, the effect of electrochemotherapy using biochanin A was investigated. For this purpose, a voltage of 800 and 1200 V/cm was used, combined with a solution of biochanin A with a value of 0.015; 0.03125; 0.0625 and 0.125 μmol/ml. Cell survival was determined by MTT after 24h or 72 hours incubation.
Results: The obtained results indicate that electroporation decreased cell survival after 24h and 72h incubation proportionally to the increasing voltage. It turns out that incubation with biochanin A alone, resulted in decreased viability of tumor cells in particularly at the concentration of 0.125 μmol/ml caused the highest cytotoxic effect. Electroporation significantly supported anticancer effect of biochanin A and contributed to the cell death of higher number of cells in comparison to EP or biochanin A applied alone.
Conclusions: The obtained results proved that an increased cell membrane permeability caused by electroporation induced enhanced transport of biochanin A to cancer cells. EP enabled for an uncontrolled flow of anticancer molecules into the interior of the cell. It turns out that incubation of cell culture with biochanin A causes the death of some cells and based on the obtained results it can be concluded that the combination of electroporation with biochanin A may prove to be an effective method of combating human colon cancer.
ACKNOWLEDGEMENTS: The research was supported by SONB.A040.17.001 project (UMO-2016/22/E/NZ5/00671, PI – J. Kulbacka, WMU).

P 3.

The influence of Ca^{2+} in combination with electroporation on drug resistant human colon adenocarcinoma cells (LoVo/DX)

D. Bocheńska[1], J. Kulbacka[2]

[1]*Chemical Department, Wroclaw University of Science and Technology, Wrocław, Poland;*
[2]*Department of Medical Biochemistry, Wroclaw Medical University, Wrocław, Poland*
e-mail: dominika.bochenska21@gmail.com and julita.kulbacka@umed.wroc.pl

Purpose: Calcium is an intracellular regulator of many physiological processes. Its concentration inside cells is highly regulated and kept at a low level. Its large abundance in the cytoplasm induces processes that lead to cell apoptosis. It is the big challenge to increase calcium content in cells. Ca^{2+} overload in cancer cells may be induced by electroporation (EP) process.
The aim of the work was to assess how Ca^{2+} contained in Ringer solution (RS) and calcium gluconate (CaG) (preparations used in medicine) affect the survival of human colon adenocarcinoma cells, that reveal drug resistance. The cells were subjected to EP and EP in combination with RS and CaG.
Materials and methods: The studies were carried out on the multidrug resistant human colon adenocarcinoma cells (LoVo/DX). Cell were exposed to differently diluted Ringer solution and calcium gluconate for 24h and 72h with. The following EP parameters were verified: voltage of the electric field – 400, 800, 1200, 1600 and 2000 V/cm (8 pulses 100μs, 1Hz). For RS and CaG delivery was selected 400 and 800 V/cm. Cell survival was checked by an MTT test after 24 and 72h incubation.
Results: Our results show, that calcium ions stimulate cells proliferation – only in case of high Ca^{2+} concentration cell viability decreased. In electroporation, the higher applied field voltage, the greater decrease of cell survival was observed. EP combined with RS or CaG induced slight decrease in cell survival, in comparison to electroporation.
Conclusions: It has been shown that calcium ions can act as an anti-cancer agent in combination with EP, thus the application of preparation as Ringer solution or calcium gluconate as a source of calcium ions can have some anticancer potential.
ACKNOWLEDGEMENTS: The research was supported by SONB.A040.17.001 project (UMO-2016/22/E/NZ5/00671, PI – J. Kulbacka, WMU).

P 4.

Cell culture and animal models as useful methods for excitotoxicity estimation

A. Brzezińska[1], A. Rorbach-Dolata[2], A. Piwowar[2]

[1] *Scientific group by the Department and Department of Toxicology, Faculty of Pharmacy with the Department of Medical Analytics, Wroclaw Medical University, Borowska 211, 50-556 Wrocław, Poland*
[2] *Department and Department of Toxicology, Faculty of Pharmacy with the Department of Medical Analytics, Wroclaw Medical University, Borowska 211, 50-556 Wrocław, Poland*
email: a.brzezinska.1994@gmail.com

In developed countries advanced health care and high living standards contribute to increased average life expectancy and percentage of senescent population. Concomitant prevalence of age-associated disorders, such as neurodegenerative diseases, necessitates the need to deeply understand molecular mechanisms underlying these diseases. Although neurodegenerative diseases are heterogeneous group of disorders with different genetic etiologies and clinical phenotypes, a growing body of scientific studies revealed, that neuronal death – a common attribute of neurodegeneration, might be induced by different processes, among which process known as excitotoxicity is getting more and more interesting. This specific type of neurotoxicity is mediated by glutamate – the most abundant excitatory amino acid neurotransmitter present in central nervous system. Excessive activation of ionotropic and metabotropic receptors by glutamate and consequent inordinate excitatory neurotransmission lead to a number of deleterious consequences, including neuronal death. Thus, deeper examination of the pathways involved in excitotoxicity is of high priority for the future clinical treatment of neurodegenerative diseases. Moreover, scientific data could indicate the link between neurodegeneration and various triggers of excitotoxicity, such as hypoglycaemia, chronic hyperglycemia and hypoxia. Therefore, the aim of the analysis is a comprehensive summary of the literature data includes methods used to examine cytotoxicity induced by glutamate in various cell culture and animal models including i.e. human neuroblastoma cell line, clonal line of human teratocarcinoma cells, isolated chicken embryo retina or rat retinal segments.

P 5.

The viability of cancer cells treated with DCA

A. Czapla[1], K.Tarasek[2]

[1]*Department of Cell Biology, Medical University of Silesia, Sosnowiec, Poland*
[2]*Department of Cell Biology, Medical University of Silesia, Sosnowiec, Poland*
email: aniaa.czapla@gmail.com

The extraordinary metabolic profile of cancer cells conducting aerobic glycolysis, might confer apoptosis resistance and be terapeutically targeted. Dichloroacetate (DCA) inhibits pyruvate dehydrogenase kinase (PDK) and shifts metabolizm from glucolysis to glucose oxidation only in cancer cells [1]. Additionally, it activates pyruvate dehydrogenase, decreases mitochondria membrane potential and increases mitochondrial H2O2. It generates many reactive oxygene`s forms, what induces apoptosis in cancer cells at the same time, not changing metabolism in normal cells. DCA causes apoptosis, decreases proliferation and inhibits tumor growth, without apparent toxicity.

The aim of the research was to evaluate the viability of cells of different tumor lines exposed to DCA. The cancer cells used in the experiment were Caco-2 (*human epithelial colorectal adenocarcinoma cells*), A549 (*human lung carcinoma cells*), MDA-MB-231 (*human breast adenocarcinoma cells*), MCF-7 (*human breast adenocarcinoma cells*), T47D (*human breast adenocarcinoma cells*), C-32 (*human amelanotic melanoma cells*), SNB-19 (*human glioblastoma cells*), Colo-829 (*human melanotic melanoma cells)*, HepG2 (*human liver hepatocellular cells*). The cellular material was incubated with DCA at a concentration of 1.5 mM to 100 mM for 48 hours. After the incubation period, the folowing tests were performed: CVDE (Aniara), WST-1 (Roche Diagnostics) and LDH (Roche Diagnostics). In the CVDE test (Aniara), the crystal violet penetrates the cell membrane and then reacts with nucleic acids. The amount of the dye absorbed by the cells is directly proportional to the number of cells. The WST-1 test (Roche Diagnostics) principle is based on the ability of living cells to break down the bright red tartrazolium salts of WST-1 into a dark red formazan, the amount of which is measured spectrophotometrically and correlates with the number of viable cells. In the LDH test, the cytosolic enzyme (lactate dehydrogenase), released into the culture medium only after damage to the cell membrane, is determined; the amount of lactate dehydrogenase is directly proportional to the amount of spectrophotometrically determined formazan and correlated with the number of dead cells. The results of all tests confirm the cytotoxic effect of DCA on the tested tumor cells.

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P 6.

Enzymatic synthesis of phospholipid biopreparations containing anisic acid

M. Czarnecka, M. Rychlicka, A. Gliszczyńska

Department of Chemistry, Wroclaw University of Environmental and Life Sciences, Wroclaw, Poland
email: marta.b.czarnecka@gmail.com

Dietary phenolic acids receive considerable interest for their presumed role in the prevention of various degenerative diseases. 4-Dimethoxybenzoic acid (anisic acid), the constituent of Chinese star anise, is one of those natural compounds which biological properties can be useful in medicine. Anisic acid can play the role of anticancer agent because effectively inhibits the activity of COX-2 [1] and exhibits the ability to reduction of oxidative stress that is one of the significant reasons in carcinogenic process [2]. Moreover, anisic acid possesses hepatoprotective activity [3] and can be applied in medical and cosmetic products also as an active substance against hyperpigmentation, which exhibits the ability to the inhibition of tyrosinase [4].
However, the use of anise acid in lipophilic preparations is limited due to its low solubility, which reduces its effectiveness and activity in human body [5]. To overcome this, in our study, we focused on the development of a biotechnological method of obtaining the phospholipids enriched with this phenolic acid. Here we present the results of enzymatic acidolysis of egg yolk phosphatidylcholine (PC) with anisic acid catalyzed by five commercially available lipases.

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P 7.

Short- and long-term adverse effects of testosterone cross-sex hormone therapy in transgender patients assigned female at birth

M. Czwojdzińska, K. Bednarska

Department of Toxicology, Wroclaw Medical University, Wroclaw, Poland
email: bednarska.katarzyna93@gmail.com

Aim: The frequency of adverse effects – especially long-term adverse effects – of testosterone intake in transgender patients assigned female at birth is relatively unknown both to patients and physicians. The aim of the study was to gather and analyse data about such effects, concentrating on the time of their occurrence. Materials and methods: The study was based on an original questionnaire addressed to transgender patients undergoing cross-sex hormone therapy (CHT) with the use of testosterone. Answers from 600 participants were gathered.
Results: The majority of research participants observed some adverse effects during the course of their CHT. Almost half of participants (48%) were taking testosterone for less than two years. In general, CHT was continuous (94%) and intramuscular injections were the most popular formulation of testosterone used (91%). Adverse effects varied in time – according to research analysis the most common short-term adverse effects were acne (71%), mood swings (38%) and cramps (29%), whereas the most common long-term adverse effects were alterations in blood lipids (37%), hypertension (12%) and cramps (10%). Long-term adverse effects were noticeably less frequent in patients who declared they were leading a healthy lifestyle with regular physical activity.
Conclusion: CHT is bound with a wide range of adverse effects. Some of them – like cramps before hysterectomy or osteoporosis in case of an inadequate dosage of testosterone – are specific for transgender patients alone. As CHT remains the only available therapy, it is crucial to inform them about the possible adverse effects and the importance of prevention

P 8.

Risk factors for chronic kidney disease (CKD) in HIV-infected patients

J. Domagała[1], Z. Marchewka[1], B. Szymańska [1], B. Knysz[2], A. Piwowar[1]

1Department of Toxicology, Medical University in Wrocław, Poland
[2]Department of Infectious Diseases, Hepatology and Acquired Immune Deficiencies, Medical University in Wroclaw, Poland
email: justyna_domagala@poczta.onet.pl and zofia.marchewka@umed.wroc.pl

Currently chronic kidney disease is more often observed in patients infected with HIV than in the past and becomes one of the significant reasons of increasing number of deaths among theses pts. In the recent years there is a constant growth of interest in research on chronic kidney disease in HIV patients. The mechanism of renal pathology in HIV patients is complex.
The aim of the study was to analyze reasons leading to the development of chronic kidney disease in HIV positive individuals receiving combined antiretroviral therapy (cART). The influence of HIV on kidneys resulting in HIVAN, thrombotic microangiopathy or HIV immune complex kidney disease-HIVICK, as well as nephrotoxicity of most often used antiretroviral drugs, components increasing drug`s bioavailability, genetic patterns influencing kidney`s pathology, were presented. Coinfections with viral pathogens such as CMV, EBV, VZV adenoviruses, HBV, HCV were also analyzed. We indicated harmful components of chemsex and other comorbidities that can cause nephropathy.
Multiplicity and diversity of presented factors prove that indeed kidney injury is a critical issue for clinicians and diagnosticians. Understanding of the reasons and monitoring systematically kidney function can allow clinicians to modify and adapt proper therapies to prevent end-stage kidney disease.
Complex patomechanism and clinical picture of HIV infection that requires complex therapy demand particularly evaluation of the renal function as the chronic kidney disease might be subclinical. This situation must not be worsening, and cART should be modified in a way to prevent end-stage kidney disease.

P 9.

Dopamine detection using fluorescence-based sensor with graphene quantum dots

K. Drzozga, J. Cabaj

Department of Chemistry, Wrocław University of Science and Technology, Wrocław, Poland
email: kamila.drzozga@pwr.edu.pl

Rapid and sensitive detection of dopamine is extremely important in modern medicine. Dopamine is very important neurotransmitter, which mainly occurs in the brain and central nervous system of mammals. Dopamine is responsible for the transmission information of moving through the nervous system and plays an important role in processes of learning or memory. Detection of dopamine is significant for diseases associated with the central nervous system such as Parkinson or schizophrenia.

Biosensor is a chemical sensor, which except of converter also possess a biologically active material, which is the basis for the detection of specific chemicals in the sample. Each biosensor device mainly consists two elements: a sensitive element, where is recognition of receptor-analyte, and a transducer element which receives the signal and converts it into a measurable signal. Through these two elements biosensors can be divided in two categories: due to the recognition element (e.g immunosensor) and due to the transducer (e.g optical sensor).

In developed optical biosensor for detection of dopamine, graphene quantum dots (GQDs) are used. In such sensor dopamine molecules coat the GQD surface – in result occurs quenching of fluorescence due to Resonance Energy Transfer (FRET). Changes in fluorescence correspond to specific concentrations of the neurotransmitter in tested sample, so it is possible to accurately determine the concentration of dopamine in the sample.

ACKNOWLEDGEMENTS: The authors thank to Wrocław University of Science and Technology for financial support (10401/0194/17).

P 10.

Comparative assessment of the antibacterial effect of selected essential oils

R. Dudek-Wicher, A. Junka, M. Bartoszewicz

Department of Pharmaceutical Microbiology and Parasitology, Medical University Piastów śląskich, Wrocław, Poland
email: r.dudek.wicher@gmail.com

In the era of increasing pathogens' resistance against synthetic drugs, use of natural substitutes is highly desirable [1]. Antimicrobial activity of 5 essential oils: geranium, cedar wood, clove, frankincense and tea tree oil were assessed against planktonic and biofilm forms of 5 bacterial strains: *Staphylococcus aureus, Enterococcus faecalis, Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae* and against one fungal strain – *Candida albicans*.
Three assessment methods were applied. Agar diffusion method showed the lowest sensitivity. In turn, micro-dilution method was more accurate, however it allowed to measure antimicrobial compounds diffused in liquid only, while it has been known that the strongest antimicrobial activities are displayed by volatile substances of essential oils [2]. Therefore, for the purposes of this study, self-designed modification of A.D.A.M. test (Antibiofilm Dressing's Activity Measurement) [3] was used, allowing examination of antimicrobial potential of volatile oils' fractions. Microbial cellulose discs, produced by *Gluconacetobacter xylinus* species, was used as an oil's carrier. Results proved that, among tested, the geranium oil's volatile fractions, displayed the highest antimicrobial effect against tested strains of pathogens.

ACKNOWLEDGEMENTS: Research was conducted by means of statutory grant no. STM.D230.17.004.

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P 11.

The impact of bionanocellulose on osteoblasts and fibroblasts cell lines

K. Dydak[1], A. Junka[1], P. Szymczyk[2], M. Bartoszewicz[1]

[1]*Department of Pharmaceutical Microbiology and Parasitology, Wrocław Medical University, Wroclaw, Poland*
[2]*Centre for Advanced Manufacturing Technologies, Wrocław University of Science and Technology, Wrocław, Poland*
email: karolina.dydak@op.pl

Introduction: Bionanocellulose (BC) is a natural polymer produced by *Komagataeibacter xylinus* bacteria. BC is characterized by high crystallinity, strength and biocompatibility, making it one of the most promising biomaterials to be used for pharmaceutical and medicinal purposes, including bone implantology. Ti-Al-Nb scaffold implants are future bone implants displaying favorable features with regard to their low cytotoxicity, durability and low weight. Coating of these implants with BC may further increase Ti-Al-Nb scaffolds' biocompatibility.
Aim: The aim of the study was to verify if coating of Ti-Al-Nb implants with BC may have a stimulating effect on the growth of osteoblasts and fibroblasts cell lines and to determine a level of cytotoxicity of such modified implants.
Materials and methods: Cytotoxicity and colonization tests were carried on three types of samples: Ti-Al-Nb scaffolds coated and uncoated with BC and bionancellulose discs (control experiment). The *K. xylinus* ATCC 53524 reference strain, the ATCC HTB-96 U-2 OS osteoblast cell line and the L-929 (NCTC) fibroblasts cell line were used. Normative cytotoxicity and colonization tests were performed in *in vitro* setting.
Results: Performed tests revealed that BC has no cytotoxic effects on fibroblasts and osteoblasts cell lines according to binding norm. For fibroblasts, the lowest cytotoxicity was observed in case of native BC samples, and for osteoblasts, in case of BC-coated scaffold samples. Both cell types grew significantly better on BC-coated implants than on uncoated implants.
Conclusion: Coating of Ti-Al-Nb implants with BC increases their applicability for replacement of bone loss.
ACKNOWLEDGEMENTS: Research was performed by means of statutory funding ST-904.

P 12.

Conjugates of phospholipids with isoprenoids and CLA as innovative anticancer agents

A. Gliszczyńska[1], M. Rychlicka[1], N. Niezgoda[1], M. Czarnecka[1], M. Świtalska[2], J. Wietrzyk[2]

[1]*Department of Chemistry, Wroclaw University of Environmental and Life Science, Wroclaw, Poland*
[2]*Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wroclaw, Poland*
email: anna.gliszczynska@wp.pl and rychlicka.magda@wp.pl

Isoprenoids are a group of natural compounds that posses a broad spectrum of biological activities in which anticancer properties of monoterpenes should be especially highlighted. Despite high biological activity and low toxicity of monoterpenes, their use is limited by their relatively short duration in the human body. Unfortunately, their rapid metabolism resulting in a lack of response after their application in therapy [1]. One of the methods of increasing the concentration of therapeutic molecules in the blood without necessity to increase their doses is to modify their structure by joining them with compounds of high bioavailability in the body. An example of such bioavailable and stable compound circulating in the bloodstream is phosphatidylcholine (PC). Many studies have shown that over 90% of consumed PC is absorbed from the digestive system and a significant part of it stays in blood even up to hundred hours after ingestion [2]. In order to obtain a pharmaceutical preparation with slow monoterpenes release in the body and their prolonged anticancer action we designed the new phospholipid molecules which contain in the *sn*-1 position isoprenoid acids and CLA isomer (*t*10, *c*12) in the *sn*-2 position. The antitumor activity of obtained derivatives has been confirmed in *in vitro* tests towards selected cancer cell lines and compared with activity of free monoterpene acids and commercially available cytostatic.

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P 13.

New view on investigations of exposure of cell lines to human biological samples

N. Głogowska[1], A. Rorbach-Dolata[2], A. Piwowar[2]

[1] *Scientific group by the Department and Department of Toxicology, Faculty of Pharmacy with the Department of Medical Analytics, Wroclaw Medical University, Borowska 211, 50-556 Wrocław, Poland*
[2] *Department and Department of Toxicology, Faculty of Pharmacy with the Department of Medical Analytics, Wroclaw Medical University, Borowska 211, 50-556 Wrocław, Poland*
email: nataliaglogowska@op.pl

The present scientific world research usually based on the use of cell culture, relies on maintaining cell culture in biochemical processes in appropriately selected and sterile conditions. Cell cultures have become an ideal alternative to controversial animal studies (in vivo). The advantage of in vitro research is the versatility of its use and obtaining consistent, reliable and reproducible results. The choice of cellular line depends on the aim of the researches. Experiments conducted on cell lines provide in-depth knowledge of morphology, cells differentiation, protein expression of macromolecules, metabolism and signal pathways of a given cell type. Both standard cell lines and lines of neoplastic cells can be used.

In vitro cultures are often used to study effects of various factors such as oxidative stress, medications, toxic substances, carcinogenic or regulatory agents. New research trends are connected with the investigation of the impact of exposure of human material on cell lines and observation of changes in the functioning of cell culture.

The purpose of this review is to analyse the latest reports on cell lines and their exposure to material of human origin. This makes it possible to evaluate the effects of their interactions and create an in vitro model more similar to in vivo conditions. This seems to be a new trend in research on cell lines.

P 14.

Anticancer effects of celastrol and resveratrol on sensitive and drug-resistant colon cancer cells

J. Gołuchowska, H. Moreira, A. Szyjka, K. Gąsiorowski

Wroclaw Medical University, Wroclaw, Poland
email: justyna.goluchowska@gmail.com and helena.moreira@umed.wroc.pl

Introduction: Celastrol (CEL) and resveratrol (RSV) are pharmacologically active plant polyphenols. Both polyphenols possess anti-inflammatory and anti-oxidant properties, inhibit cell proliferation, induce cell cycle arrest and apoptosis in several cancer cell models. We evaluated the effects of CEL and RSV on apoptosis and on cell cycle in both sensitive and doxorubicin resistant colon cancer cells.
Methods: Human colon cancer cells both sensistive (Lovo) and resistant to doxorubicin (Lovo/DX) were incubated for 18 hours with CEL or RSV (1-10 μM). Apoptotic cells frequency was estimated after staining with AnnexinV-Alexa488 / PI and cell cycle analysis was done by PI staining method. Flow cytometric measurements were carried out with CyFlow®Space cytometer (Sysmex-Partec) equipped with FlowMax software. Cell cycle analysis was done with MultiCycle™DNA analysis model.
Results: CEL and RSV caused an increase of cells in the S phase and marked decrease of cell population in the G2/M phase, both in Lovo and Lovo/DX cultures. CEL caused significant, dose-dependent increase of apoptotic cell frequency in Lovo/DX cultures, while in Lovo cell cultures it predominantly induced necrosis. RSV caused an increase in the percentage of cell undergoing apoptosis in Lovo cells only.
Conclusion: Both polyphenols, CEL and RSV, demonstrate anticancer effects on colon cancer cells, by induction of apoptosis and cell cycle arrest. Their proapoptotic effects vary depending of the cells sensitivity to cytostatics. However, our data suggests, that celastrol and resveratrol might be useful in supporting the treatment of colon cancers.
ACKNOWLEDGEMENTS: This work was supported by Wroclaw Medical University Grant Nr. ST.D130.16.009.

P 15.

Edible Honeysuckle Berries (*Lonicera caerulea* var. *kamtschatica* Sevast.) – a Promising Source of Antioxidants

M. Gołba, A. Sokół-Łętowska, A. Z. Kucharska

Department of Fruit, Vegetable and Plant Nutraceutical Technology, Wrocław University of Environmental and Life Science, Chełmońskiego 37, 51-630 Wrocław, Poland
email: anna.sokol-letowska@upwr.edu.pl

Edible honeysuckle berries (*Lonicera caerulea* L. var. *kamtschatica* Sevast.; Caprifoliaceae family) become increasingly popular mainly due to their antioxidative, antibacterial and anti-inflammatory properties which are being intensively studied. The mentioned features are the effect of high content of polyphenols and iridoids in the fruit. Although still not fully analyzed, honeysuckle berries have a high potential to be used in food and pharmaceutical industry having a positive influence on human health. In this study antioxidant activity of the fruit was investigated using DPPH (1,1-Diphenyl-2-picrylhydrazyl radical), FRAP (ferric reducing ability of plasma) and ABTS (2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)) methods. The results obtained indicate that honeysuckle berries could serve as an efficient antioxidant supporting human health and acting against diseases.

P 16.

The biomolecular interactions of lysosomotropic surfactants with lysozyme and its effect on the conformation of protein: a biophysical approach

K. Hyjek, Ż. Czyżnikowska, T. Janek

Department of Inorganic Chemistry, Wroclaw Medical University, Wroclaw, Poland
email: zaneta.czyznikowska@gmail.com and tomasz.janek@umed.wroc.pl

Given the growing interest of new biomedical applications for lysosomotropic surfactants, the aim of this work was to present a detailed molecular study of the interactions between cationic surfactants and lysozyme, in order to characterize the basic features of these compounds and the potential application of lysosomotropic surfactants in medicine and drug delivery. The mechanism of the interactions between surfactants, namely, (2-dodecanoyloxyethyl) trimethylammonium bromide (DMM-11), (2-dodecanoyloxypropyl) trimethylammonium bromide (DMPM-11), (2-pentadecanoyloxymethyl) trimethylammonium bromide (DMGM-14) and lysozyme was investigated by fluorescence and circular dichroism (CD) spectroscopy. The binding sites of the molecular systems in lysozyme have been placed using molecular docking procedure.

The results showed that the fluorescence changing of the tryptophan of lysozyme by lysosomotropic surfactants was attributed to the formation of the protein-surfactant complexes. The CD results showed conformational changes in the secondary structure of lysozyme by the addition of lysosomotropic surfactants. Moreover, the molecular docking study suggested that hydrogen bonding play a key role in the protein-surfactant binding.

ACKNOWLEDGEMENTS: The authors would like to kindly thank Dr. Jacek Łuczyński for the opportunity to carry out research on the substances synthesized by him.

P 17.

Influence of divalent counterions on conformational changes and self-assembly of lipopeptide amphisin

T. Janek[1], L. R. Rodrigues[2], Ż. Czyżnikowska[1]

[1]*Department of Inorganic Chemistry, Wroclaw Medical University, Wroclaw, Poland*
[2]*Centre of Biological Engineering, University of Minho, Braga, Portugal*
email: tomasz.janek@umed.wroc.pl

The present study aimed to explore the interactions of divalent counterions with biosurfactant amphisin using circular dichroism (CD), ultraviolet-visible (UV–Vis) and density functional theory (DFT). The conformational analysis of metal-amphisin complexes provided detailed information on the metal – lipopeptide binding sites. The results showed that Cu^{2+} is coordinated by three nitrogen atoms and one oxygen atom of the aspartic acid side chain, whereas Mg^{2+}, Ca^{2+} and Zn^{2+} favor the association with oxygen atoms spanning the lipopeptide backbone. On the other hand, the aggregation and self-assembly of amphisin induced by divalent metal ions was studied by dynamic light scattering (DLS). Our results revealed that the self-assembly process of amphisin can be controlled by the addition of metal ions. The analysis of CD spectra revealed the occurrence of changes in the secondary structure of the amphisin when exposed to doubly charged metal cations. Finally, quantitative structure-activity relationship (QSAR) results indicated that metal-amphisin complexes have very good biological activities and exhibit properties such as anti-adenovirus, anti-herpes simplex virus, antioxidant and anti-cancer activity. In conclusion, understanding the interactions between divalent counterions and amphisin may influence its potential applications in medicine.

ACKNOWLEDGEMENTS: This work was supported by Polish-Portugal Executive Program for years 2017-2018.

P 18.

New view on association of xenoestrogens with male reproductive potential

K. Jurkowska[1], E. M. Kratz[2], E. Sawicka[3], A. Piwowar[3]

[1]*Student Science Club of Department of Toxicology, Faculty of Pharmacy, Wroclaw Medical University, Wroclaw, Poland*
[2]*Department of Chemistry and Immunochemistry, Faculty of Medicine, Wroclaw Medical University, Wroclaw, Poland*
[3]*Department of Toxicology, Faculty of Pharmacy, Wroclaw Medical University, Wroclaw, Poland*
email: k.jurkowska93@o2.pl

In the last decade, as documented in many reports, the increased reduction of male fertility, manifested by the decreased number of spermatozoa in the ejaculate, abnormality in their morphology and reduced motility was observed. The significant role of disturbed oxidative-antioxidative balance and proteases-inhibitors equilibrium in seminal plasma was also discussed by some authors. Human reproductive problems may be also associated with different natural or synthetic chemical substances present in the environment. They can act as estrogens, mainly via estrogen receptors, and they are called xenoestrogens. Physiologically estrogens are steroidal sex hormones found in high concentrations in women of childbearing age. However, in men a high level of these hormones may be also observed, especially in the male reproductive tract. It is important that xenoestrogens have an impact not only on the male reproductive potential, but also on the development and proper functioning of other human tissues. The participation of xenoestrogens in the disturbances of women's health is well known. They participate in pathogenesis and cancer development, especially breast or ovarian carcinoma is proved. However, there is little known about their participation in male reproductive health. Based on the analysis of available information we would like to pay attention on xenoestrogens as factors contributing in male infertility and/or decreased fertlity, which role are underestimated. Considering the increasing number of men with fertility problems in the societies of developing countries, xenoestrogens often used in industry and agriculture, seem to play an important role in the `decreased male fertility.

P 19.

Evaluation of biological features of Mesenchymal Stem Cells growing on biomaterial in 3D configuration

I. Kamińska, A. Lis-Nawara, J.K. Bar

Department of Immunopathology and Molecular Biology, Wrocław Medical University, Wrocław, Poland
email: iwona.kaminska@umed.wroc.pl

Stem cells in human body are responsible for regeneration of tissue damage. The unique features of adult mesenchymal stem cells (MSC) such as differentiation into osteoblast, chondrocytes, nerves cells allow to use its in regenerative medicine. MSC can be injected directly into the tissue defect or be seeded on membranes as a 3D construction.
The aim of our study was to evaluate MSC proliferation and its adhesive ability in 2D and 3D *in vitro* culture.
The cell line of MSC was cultured on culture bottles (2D) for 7, 14 days and on membrane (3D) HYAFF-11 (benzylic ester of hyaluronic acid) for 7 days. The stem cells markers expression in 2D were analyzed by monoclonal antibodies recognize: CD44, CD90, CD105, CD133 and proliferation activity by Ki67 antigen. The adhesive ability of MSC in 3D was evaluated by DAPI staining.
In 2D culture no changes in expression of analyzed stem cell biomarkers, proliferative activity after 7, 14 days were observed. MSC showed very good adhesion to culture bottles. Similar results were observed when MSC grew on HYAFF-11. The microscopic analysis of scaffold showed the presence of MSC cells on fibers of 3D structure. The analysis of released MSC from scaffold showed that they do not loose proliferation activity. Our results revealed very good adhesion of MSC to the scaffold. Our results showed that bioimplant consist of the HYAFF-11 and MSC may be a useful biomaterial to regeneration of tissue defects.
ACKNOWLEDGEMENTS: This study was supported by Wroclaw Medical University grant no. STM.B132.17.012.

P 20.

The influence of curcumin on human Glioblastoma multiforme SNB19 cells

P. Wawryka, A. Kiełbik, D. Przystupski

Department of Medical Biochemistry, Chałubińskiego 10, 50-368 Wrocław, Poland

email: akielbik6@gmail.com

Background: Glioblastoma multiforme (GBM) is a highly invasive (WHO grade IV) brain tumour that has a very poor prognosis for patients (median survival 14.2 months). In the study we treated glioblastoma multiforme with curcumin (CUR), as it was proved to reduce proliferation of several types of cancer before. Curcumin, polyphenol compound extracted from turmeric, exerts anti-proliferative and apoptotic effects via multiple molecular targets, changes activity of various enzymes and increases reactive oxygen species production in cancer cells.
Objectives: Main aim of the study was to measure and analyze the cytotoxic effect of curcumin on gioblastoma cells after and define the optimal concentration, which affect the tumor cells.
Material and Methods: The research was performed on Glioblastoma cell line SNB19. The cells were grown in sterile condition in culture bottles as a monolayer. The SNB-19 cells where incubated in different concentration of curcumin. The mitochondrial activity was measured by MTT cytotoxicity assay after 24h and 48h.
Results: The curcumin treatment significantly decreased cell viability compared with untreated cells. After incubation for 24 h, CUR remarkably reduced cell viability in higher concentrations, whereas after 48h incubation, a decent viability decrease was observed also in medium and lower concentrations.
Conclusions: As results show, accordingly chosen parameters eliminate glioblastoma cells. We conclude that curcumin may have a positive influence on tumor therapy. Futher research on glioma cells and normal cells are required to get more informations about direct cellular effect and side effects of curcumin.

The work was created as part of the activity of the student scientific club "Cancer cell biology" (SKN No. K149).

P 21.

MetC and GceC – two different strategies of purification

M. Kolonko

Wroclaw University of Science and Technology, Wroclaw, Poland
email: martakolonko@gmail.com

Methoprene tolerant (Met) protein is known as an D. melanogaster juvenile hormone (JH) receptor. Met binds JH and is important for the function of hormone in preventing the precocious differentiation during development. The deletion of met gene is lethal to most species of insects. However, in D. melanogaster exists Met paralog – Germ cell-expressed protein (Gce), capable of ensuring the survival of Met null mutants. However, some of Met and Gce functions are not fully redundant and proteins exhibit tissue specific distribution.

Bioinformatic analyses assign Met and Gce to the family of bHLH-PAS (basic helix-loop-helix/Per-Arnt-Sim) transcription factors. The bHLH-PAS family members contain three characteristic and highly homologous domains, bHLH, PAS-A and PAS-B, responsible for dimerization and specificity. The similarity between the Met and Gce primary structures is limited to these defined domains, while their C-terminal fragments (MetC, GceC, respectively) show significant differences. In addition, the results of in silico analysis indicate that MetC and GceC may exhibit properties of intrinsically disordered regions (IDRs).

The structural differences between the C-terminal fragments of Met and Gce could be crucial for their function and subcellular localization distinction during D. melanogaster development. Despite their homology, two extremely different strategies have been used to obtain the homogenous MetC and GceC preparation. MetC purification process was conventional and efficient. The protein is soluble and stable. During the GceC overexpression, we faced a number of difficulties related to the expression efficiency and protein insolubility. Finally, we decided to apply the technique allowing the protein refolding from inclusion bodies.

P 22.

Substances isolated from snake venoms as a new perspective in melanoma treatment

K. Kotowski

Department of Medical Biochemistry, Wroclaw Medical University, Chałubińskiego 10, 50-368 Wroclaw, Poland
e-mail: Krzysztof.kotowski@student.umed.wroc.pl

Melanoma is the most aggressive form of skin cancer, with an increasing incidence in the last 30 years and with an intrinsic metastatic and chemoresistance potential that is responsible for its poor prognosis and survival after diagnosis [1, 2]. Despite the recent promising news from the field of immunotherapy, there is an urgent need for new therapeutic approaches that are free of resistance mechanisms and side effects. [3] Due to this fact scientists are looking for new substances, which may within few years time become a new alternative for classic treatment.

Very interesting proposal are substances which are of natural origin, especially isolated from snake venoms. One of these substances is disintegrin Labein isolated from *Macrovipera labetina*, which shown antiproliferative activity through extracellular signal-regulated kinase on the human melanoma cell lines SK-MEL-28 and LU-1205. The next one is Colombistatin, an RGD disintegrin isolated from *B. colombiensis* venom. Studies on this disintegrin show that r-Colombistatin 2,3 and 4 inhibited SK-Mel-28 adhesion to collagen I. Also, very interesting potentially anticancer substance is BthTX-I (which has been shown to be cytotoxic for the murine (PC-12 and B16F10) tumor cell lines by inducing the cell death mechanisms of apoptosis and/or necrosis. Additionally, BthTX-I was able to promote delay in the G0/G1 phase of the cell cycle of murine tumor cells. [4]

This work was aimed to comprehensively summarize all discoveries from recent years regarding substances originating from snake venom, which strengthens the potential use of them as an anti-neoplastic drug against melanoma.

ACKNOWLEDGEMENTS: The study was created as part of the work of the Students Scientific Group of "Biology of Cancer Cell" at the Medical University of them. Piastów Śląskich in Wroclaw.

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P 23.

Optimizing spectral and spatial denoising techniques for IR Imaging

P. Koziol[1], J. Skibinska[1,2], M. Juszczyk[1,3], S. Urbaniak-Wasik[4], C. Paluszkiewicz[1], W. M. Kwiatek[1], T. P. Wrobel[1*]

[1]Institute of Nuclear Physics Polish Academy of Sciences, PL-31342 Krakow, Poland
[2]Faculty of Electrical Engineering, Automatics, Computer Science and Biomedical Engineering, AGH University of Science and Technology, Mickiewicza 30, Krakow, Poland
[3]Faculty of Physics and Applied Computer Science, AGH University of Science and Technology, Mickiewicza 30, Krakow, Poland
[4]Pathology Department, Jagiellonska 70, Kielce, Poland
email: tomaszpwrobel@gmail.com,
tomasz.wrobel@ifj.edu.pl

INTRODUCTION: Despite the fact that Fourier-Transform Infrared Spectroscopy (FTIR) is one of the least noisy of vibrational spectroscopy techniques, achieving high Signal to Noise Ratio (SNR) still requires performing multiple scans. However, acquisition time can be significantly shortened by applying denoising algorithms with properly chosen parameters[1]. The aim of this study was to investigate and optimize spectral and spatial denoising techniques such as Savitzky-Golay, Fourier Transform and Spatial Filters, based on FTIR spectra and images of a tissue specimen and simulated data.

METHODS: Measurements were performed in transmission mode with Bruker Vertex70v Spectrometer combined with Hyperion 3000 microscope with an MCT FPA 64x64 detector and 15x and 36x objectives (pixel size of 2.7 and 1.1 micrometers, respectively). Pre-processing was done in Matlab software using built-in and home written routines. Results obtained for each technique were evaluated using SNR and Signal Distortion (SD), which were calculated for spectra as well as images.

RESULTS AND DISCUSSION: Applying different denoising techniques in FTIR data pre-processing resulted in most cases in multiple SNR increase. Optimal parameters for each method were determined by compromising between SNR and SD level. Best algorithms for spatial and spectral denoising were selected based on the juxtaposition of all results. This approach allows achieving sufficient measurement speed throughput while preserving the data quality and is implementable in other vibrational spectroscopic techniques.

ACKNOWLEDGEMENTS: This work is a part of the „Pancreatic cancer comprehensive histopathology based on IR" project, which is carried out within the Homing programme of the Foundation for Polish Science co-financed by the European Union under the European Regional Development Fund. It was performed using equipment purchased in the frame of a project co-funded by the Małopolska Regional Operational Programme Measure 5.1 Krakow Metropolitan Area as an important hub of the European Research Area for 2007-2013, project No. MRPO.05.01.00-12-013/15.

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P 24.

The influence of modified cyclodextrin on the anthracycline interaction with DNA

A. Krzak[1], O. Święch[1], M. Majdecki[2], R. Bilewicz[1]

[1]*Faculty of Chemistry, University of Warsaw, Warsaw, Poland*
[2]*Institute of Organic Chemistry, Polish Academy of Sciences, Warsaw, Poland*
email: agata-krzak@wp.pl

Anthracycline antibiotics (ANT), are most commonly used anticancer agents for the treatment of several types of cancers, such as lung, breast, prostate, brain cancers. Clinical effects of the drugs are connected with modification of the DNA structure primarily through intercalating complexes and covalent bonding [1,2]. However, the clinical application of ANT has been limited by serious adverse effects. The specific toxicity is due to generation of excess reactive oxygen species (ROS) produced in redox reactions of anthracyclines. The toxicity can be reduced by creating an inclusion complex between the anthracycline molecule and cyclodextrins (CD). The limitation in the use of CD as a carrier of anthracycline drugs is the low stability constant of the complex compared with that of the drug-DNA complex. However, appropriate modification of cyclodextrin can increase stability constants of the CD-drug inclusion complex [3].

In this study we examine the stability constant of modified CD-ANT complexes in physiological pH 7.4 and at pH 5.5 (characteristic for pathologically changed cells). Electrochemical studies revealed that at pH 7.4 the modified CD-drug complexes are stronger than at pH 5.5. We investigate the effects of the modified CD-drug complex on the anthracycline interaction with calf thymus double stranded DNA. Voltammetric and spectroscopic studies show that newly synthesized nontoxic derivatives of β-cyclodextrin containing lipoic acid, leads to an increase of drug affinity towards DNA.

ACKNOWLEDGEMENTS: This work was supported by grant 2014/13/B/ST5/04117 from NSC.

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P 25.

Scavenger receptors as lipoprotein and advanced glycation end products binding proteins in the arterial wall

A. Kuzan[1], E. Królewicz[1], K. Nowakowska[2]

[1]*Department of Medical Biochemistry, Wroclaw Medical University, Wrocław, Poland*
[2]*Department of Physiology, Wroclaw Medical University, Wrocław, Poland*
email: aleksandra.kuzan@umed.wroc.pl

Scavenger receptors are mostly known as a protein mediating uptake by macrophages covalently modified lipoproteins (e.g., acetylated, oxidized) with low density (SRA) and high density (SRBI). SRA binds to a wide variety of ligands, including, in addition to the aforementioned modified LDL, some native proteins, i.e. apolipoprotein A-I and E4, heat shock protein, CRP, some denatured or glycated proteins, as well as polysaccharide sulphates, some proteoglycans or glycolipids. SRBI seems to have an affinity for fewer compounds, but the most important seems to be HDL. While numerous studies focus on the role of this protein in the reverse transport of cholesterol in the arterial wall, the aspect of glycation products binding is neglected. In the present study, the content of various advanced glycation receptors, including SRA1 and SRBI in arterial walls was analyzed using ELISA and immunohistochemical techniques. Our observations show that the main site of SRA expression are macrophages, but certain amounts of antigen are also present in some epithelial cells and fibroblasts. The concentration of antigen can be observed in the atheromatous plaque, but it also appears in the adventitia. SRBI is expressed in the walls of blood vessels, especially in the endothelium, myocytes and macrophages, as well as in a foam cells and in the fatty core. It should be verified that SRA contributes to the development of atherosclerosis and SRBI acts as the anti-atherogenic factor.

ACKNOWLEDGEMENTS: This work was financed from grant funds for Young Scientists of the Wroclaw Medical University number STM.A.040.17.040.

P 26.

The association of copper-zinc superoxide dismutase activity with major implications of diabetes mellitus

Ł. Lewandowski, M. Kepinska, H. Milnerowicz

Department of Biomedical and Environmental Analyses, Faculty of Pharmacy with Division of Laboratory Diagnostics, Wrocław Medical University, Borowska 211, 50-556 Wrocław, Poland
email: shoo12370@gmail.com

Diabetes has been associated with oxidative stress in series of studies, carried out on animals or diabetic patients. Interestingly, copper-zinc superoxide dismutase (Cu,Zn-SOD), one of the most vital enzymatic antioxidants, appears to play a major role in tissue protection from hyperglycemia-induced oxidative damage.

Research characterizes diabetic patients of decreased Cu,Zn-SOD activity in erythrocyte lysate, probably due to enzyme glycation. Cu,Zn-SOD has a protective function for endothelial tissue, restoring proper: NO production and proliferative capacity in cells, as proven on glucose-stressed endothelium progenitor cells. Cu,Zn-SOD completely restores vascular reactivity to acetylcholine in a rabbit model of hyperglycemia. Overexpression of *SOD1* gene prevents from diabetes-induced heart defects, and Cu,Zn-SOD serum concentration correlates with cardiovascular risk in diabetic patients. Proper Cu,Zn-SOD activity has a nephroprotective function, attenuating renal injury in diabetes-induced nephropathy. Moreover, hyperglycemia-induced cataract is characterized by lower Cu,Zn-SOD concentration in lenses, compared to senile cataract control specimen.

The aforementioned research shows the importance of Cu,Zn-SOD in tissue protection in hyperglycemic conditions. Recent analysis of various Cu,Zn-SOD polymorphisms seems to support this thesis. Arg/Arg genotype of *SOD3* rs1799895 has been associated with higher risk of diabetic neuropathy development in patients of Russian origin. Research into a gene polymorphism in the promoter of *SOD3* gene, rs2284659, has been associated with myocardial infarction and all-cause mortality in diabetic patients. Further research into this matter may help assess the risk of hyperglycemia implications, potentially affecting the treatment strategy in diabetic cases.

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P 27.

Fucosylation and sialylation pattern of human milk immune glycoproteins over milk maturation stages

J. Lis-Kuberka[1], Z. Sycz[1], M. Berghausen-Mazur[2], M. Orczyk-Pawiłowicz[1]

[1]*Department of Chemistry and Immunochemistry, Wrocław Medical University, Wrocław, Poland*
[2]*1st Department and Clinic of Gynecology and Obstetrics, Wrocław Medical University, Wrocław, Poland*
email: magdalena.orczyk-pawilowicz@umed.wroc.pl

Human milk is a rich source of bioactive glycoconjugates such as free oligosaccharides, glycoproteins and glycolipids, which constitute an essential element of innate immunity passed to the newborns during breastfeeding. Glycoproteins of human milk, due to attached N- and O-glycans with sialylated and/or fucosylated glycotopes, take part in the protection of the newborn and infant from bacterial and viral infections. The most abundant human milk glycoproteins, lactoferrin (LF) and secretory immunoglobulin A (S-IgA), are known to elicit antipathogenic properties. Moreover, the fucosylated and/or sialylated glycans of S-IgA are considered as a link between innate and acquired immunity.

The aim of the study was to compare the fucosylation and sialylation profile of the lactoferrin and secretory component of immunoglobulin A (SC) during milk maturation stages, namely colostrum, transitional milk, and early and late mature milk. The expression of α1,2-/α1,3-/α1,6-fucosylated and α2,3-/α2,6-sialylated glycotopes was analyzed by the semi-quantitative lectin-based method.

The expression of α1,2- and α1,3-fucosylated glycotopes on lactoferrin and the secretory component of IgA was not related to milk maturation stages. However, in late mature milk a slight decrease to 86% and 92% of the initial level, respectively, was observed. In contrast, the α1,6-fucosylation was related to lactation stage and significantly decreased in early and late mature milk, reaching 69% and 60% of the initial level, respectively. The α2,3- and α2,6-sialylation level was almost unchanged over early lactation but in late mature milk reached 85% and 75%, respectively. Detailed characterization of fucosylation and sialylation patterns of immune glycoproteins in relation to milk maturation stages can provide additional guidelines for milk banking.

ACKNOWLEDGEMENTS: This work was supported by the Medical Faculty (No STM.A070.17.060), Wrocław Medical University (Poland).

P 28.

The role of oxicam derivatives structure in their interaction with model membranes

J. Maniewska[1], J. Gąsiorowska[2], B. Szczęśniak-Sięga[1], K. Michalak[2]

[1]*Department of Chemistry of Drugs, Wroclaw Medical University, Borowska 211, 50-556 Wrocław, Poland*
[2]*Department of Biophysics, Wroclaw Medical University, Chałubińskiego 10, 50-368 Wrocław, Poland*
email: jadziasow@interia.pl

Oxicams (e.g. piroxicam, meloxicam), which are known as a class of non-steroidal anti-inflammatory drugs (NSAIDs), are mainly used in the treatment of inflammation and pain which occur during chronic rheumatic diseases. Increased expression of the COX-2 protein, which is the target of NSAIDs, has been widely reported in pathogenesis of most solid tumors, and for this reason, those drugs are evaluated also as cancer preventive compounds [1]. In this regard, Peetla *et al.* proved that biophysical changes in membrane phospholipids in multidrug resistance (MDR) of cancer cells influence the transport and delivery of anticancer drugs [2]. Moreover, Chakraborty *et al.* have demonstrated that the permeabilization of the mitochondrial membrane induced by piroxicam may promote apoptosis [3]. Thus, these results indicate that the alteration of membrane properties by NSAIDs may be an additional mechanism for their anticancer activity. Consequently, understanding the relationship between biophysical aspects of the cell membrane in drug delivery processes and drug-resistance mechanisms may be crucial to overcome the phenomenon of MDR.

The purpose of the present work is to assess the ability of newly synthesized oxicam analogues to interact with the lipid bilayers. As demonstrated in both the calorimetric and spectroscopic studies, the new oxicam analogues interact with the model membranes under consideration. In this regard, the ability of the newly synthesized oxicam derivatives to interact with the membranes depending on the details of the chemical structure of the studied compounds was confirmed.

ACKNOWLEDGEMENTS: This work was financed by the Ministry of Science and Higher Education funds for Wroclaw Medical University, project No ST-D070.17.026.

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P 29.

The role of 2-methoxyestradiol in oxidative stress induced by chromium compounds

L. Michalewska[1], N. Głogowska[2], E. Sawicka[3], A. Piwowar[3]

[1]*Department of Drugs Technology, Faculty of Pharmacy, Wroclaw Medical University, Wrocław, Poland*
[2]*Students' Science Club of Department of Toxicology, Faculty of Pharmacy, Wroclaw Medical University, Wrocław, Poland*
[3]*Department of Toxicology, Faculty of Pharmacy, Wroclaw Medical University, Wrocław, Poland*
email: ewa.sawicka@umed.wroc.pl

The increasing incidence of estrogen-dependent cancer justifies research on the effects of environmental factors and their influence on estrogen-dependent processes. The aim of study is to evaluate the role of metabolite of 17β-estradiol (2-methoxyestradiol) upon exposure to environmental toxins belonging to metalloestrogens (trivalent and hexavalent chromium). The interaction of estrogens with environmental toxins in free radicals generation (ROS), which participates in cancerogenesis, is not yet recognized. Chromium(VI) is widely present in environment. One of its toxicity pathway is free radicals' generation. Estrogens have the ability to scavange free radicals but may also act as prooxidants. Both chromium(VI) and estrogens are classified by IARC as carcinogens, so synergistic effect seems very danger. 2-Methoxyestradiol belongs to estradiol metabolites with potential protective effects in carcinogenesis.
The aim of this study was to examine the effect of 2-methoxyestradiol on the parameter of oxidative stress *in vitro* in the exposure to chromium III and chromium VI. Lipid peroxidation was measured by the level of malonyldialdehyde (MDA). The source of Cr (III) and Cr (VI) were respectively: $CrCl_3$ $6H_2O$ (Riedel de Haën) and K_2CrO_4 (POCh).
Our study demonstrated that 2-$MeOE_2$ causes a decrease in the level of erythrocyte MDA to the control in each concentration. After exposure to Cr III and VI compounds – which alone induced lipid peroxidation – 2-$MeOE_2$ lowered MDA concentration in each sample tested in combined action. Concluded, it suggests the protective effect of 2-$MeOE_2$ in exposure to environmental metalloestrogens – chromium compounds.

P 30.

Primary cell cultures – a useful tool or a dangerous trap?

O. Michel[1], D. Przystupski[1], N. Niedzielska[2], K. Bieżuńska-Kusiak[1], J. Saczko[1], J. Kulbacka[1]

[1]*Department of Medical Biochemistry, Wroclaw Medical University, Wroclaw, Poland*
[2]*Department of Biomedical Engineering, Wroclaw University of Technology, Wroclaw, Poland*
email: wysockaom@gmail.com

Cell cultures are believed to be a powerful research tool, finding its applications in various fields of science. Ever since Alexis Carrel, the Nobel Prize laureate, established cell culture of chick heart fibroblasts [1], the scientific race for the far-reaching discoveries has begun. The problem is, that Carrel had proven "beyond doubt" that his cells can live for almost infinite period of time, which was impossible to replicate in other laboratories. The addition of fresh cells appeared to be the answer to the mystery [2,3], but whether it had been a simple mistake or deliberate fraud remains an open question. Although Carrel's misleading conclusion was subsequently exposed by other scientists, the example of the world-famous deception by Haruko Obokata and her Stap cells or the case of Hwang Woo Suk who has supposedly cloned human embryonic stem cells show that a large threat can be related to unreliable and careless wet lab research [4]. This work is an overview of the history and practices in the cell culture laboratories. We will analyse the successes and failures in this scientific field and based on that and our own experience, we will present the pros and cons of using this model in modern research laboratories.

ACKNOWLEDGEMENTS: This work was supported by funds from the project STM.A040.17.039.

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P 31.

Influence of neomycin on 1,25D_3-MARRS in human colorectal carcinoma LoVo and HT-29 cells

K. Mielko[1], J. Trynda[1], A. Kutner[2], J. Wietrzyk[1]

[1]*Department of Experimental Oncology, Ludwik Hirszfeld Institute of Immunology and Experimental Therapy of the Polish Academy of Sciences, Wroclaw, Poland*
[2]*Pharmaceutical Research Institute, Warsaw, Poland*
email: k.mielko@op.pl and justynatrynda@wp.pl

The 1,25D_3-MARRS is a member of the protein disulfide isomerase family, mainly present in the endoplasmic reticulum, where it is involved in the formation of the disulfide bonds of nascent proteins and in chaperoning their correct folding. These molecules have been also found in the cytosol and the nucleus, where its binding to specific sites of DNA in the nucleus, suggest that it is also involved in the regulation of gene expression [1]. The steroid hormone 1α,25$(OH)_2D_3$ is capable of regulating gene expression through binding to its nuclear receptor (vitamin D receptor; nVDR) but can also activate a fast response pathway through 1,25D_3-MARRS interaction. A recent study confirm that this protein consists the domain which is involved in 1α,25$(OH)_2D_3$ binding [2]. Knockdown of 1,25D_3-MARRS in MCF-7 breast cancer cells reveals increased cellular sensitivity to vitamin D-mediated anti-proliferative signaling [3]. There is known that a variety of inhibitors of PDI family has already been described, particularly among peptides and antibiotics. One of the most active inhibitors is vancomycin and neomycin [4]. The purpose of this study was to examine whether 1,25$(OH)_2D_3$ biological activity is determined by neomycin inhibition of 1,25D_3-MARRS in human colorectal carcinoma LoVo and HT-29 cells.

It was observed, that the sensitivity to calcitriol and its analogue tacalcitol changed after incubation with neomycin. The 1,25D_3-MARRS mRNA and protein level was changed after treatment with calcitriol and tacalcitol combined with neomycin.

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P 32.

Synergistic effects of chamomile and lavender essential oils for antibacterial activity

M. Yau Dahiru

Department of Science Laboratory Technology, Hussaini Adamu Federal Polytechnic, Kazaure. Jigawa State Nigeria
email: mydkaz@gmail.com

Essential oils have been used for extensive applications of variety of wellness throughout documented history. With massive advancement in science, essential oils today have undergone numerous refining methods to give an improved effect. Determination of the antibacterial activities alone and in combination of lavender and chamomile essential oils is what the researched study explained. Three laboratory strains of cultured bacterial (*Pseudomonas aeruginosa,* ATCC 27858; *Staphylococcus aureus,* ATCC 6538 and *Escherichia coli* ATCC), were used in this analysis. The stock cultures were maintained at −20ºC and the sub-cultured onto Tryptone Soya agar (TSA) was incubated at 37ºC for 24 hours. The fractional inhibitory concentration index (FIC) was used in determining the oils interaction. The FIC was calculated by dividing the minimum inhibitory concentration (MIC) value of the combined essential oils with the MIC value of each essential oil placed in the combination. The ΣFIC was calculated by adding these two values. Lavender oil showed the greatest antimicrobial effect, with the lowest MIC values of 2mg/mL for both *Pseudomonas aeruginosa* and *Escherichia coli* and 4mg/mL for *Staphylococcus aureus* pathogens compared to chamomile oil when used individually. The combination of chamomile and lavender essential oils in various ratios indicated synergistic effect for all the nine ratios analysed. The minimum inhibitory concentration analysis indicated that these oils have favorable antimicrobial interactions when in combination, that are 100% and 70.4% synergistic and additive effects for the oils selected and this will offer potential for the common use of combining oils in achieving a greater therapeutic result.

P 33.

Determination of chosen advanced glycation end products in systemic lupus erythematosus patients

A. Nowak[1], B. Przywara-Chowaniec[2], A. Damasiewicz-Bodzek[1], B. Kumaszka[3], K. Tyrpień-Golder[1]

[1]Medical University of Silesia in Katowice, Faculty of Medicine with Dentistry Division in Zabrze, Department of Chemistry, Jordana 19, 41-808 Zabrze, Poland
[2]Medical University of Silesia in Katowice, Faculty of Medicine with Dentistry Division in Zabrze, 2nd Department of Cardiology, C. Skłodowskiej 10, 41-800 Zabrze, Poland
[3]District Hospital Im. Prałata J. Głowatzkiego in Strzelce Opolskie, Opolska 36A, 47-100 Strzelce Opolskie, Polska
email: agnieszka00nowak@gmail.com and ktyrpien@wp.pl

Protein glycation relates to a complex, multistage and spontaneous process, which affects both proteins present in living organisms and in vitro, in processed food. These reactions are non-enzymatic and require the presence of reducing sugars. In human body, the glycation occurs in physiological conditions and, to a greater extent, in pathological conditions. One of the diseases linked to the intensification of the glycation process is systemic lupus erythematosus (SLE) [1]. It is an autoimmunological disorder characterised by presence of autoantibodies against nucleic components of the cells. It was confirmed, that AGEs accumulate in tissues and blood plasma of SLE patients [1, 2], while data on pentosidine concentrations vary among authors [3, 4]. The disease affects the concentration of the receptor for AGEs (RAGE) too [2, 5].
The aim of the study was to evaluate concentration of chosen advanced glycation end products (AGEs) and pentosidine in patients with systemic lupus erythematosus and to compare obtained data to the control group.
The local bioethics committee's agreement was obtained to conduct the study. Blood samples were collected from 30 patients with SLE. After appropriate preparation the samples were stored in -80^{o}C until further evaluation. OxiSelect^{TM} Advanced Glycation End Product (AGE) Competitive ELISA Kit (Cell Biolabs, Inc.) and PTD (Pentosidine) ELISA Kit (Elabscience) were used to perform the experiment. Obtained data were estimated by statistical analysis in Statistica 12 software. Statistically significant difference was observed between SLE patients and control group in sera AGEs concentration ($p<0,001$), while there was no difference between these groups in pentosidine concentrations.

ACKNOWLEDGEMENTS: This work was supported by the KNW-1-038/K/7/O project Medical University of Silesia in Katowice.

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P 34.

N-alkoxyphenyl-hydroxynaphthalenecarboxamides as new photosystem II inhibitors

A. Nowok, P. Kuś

Institute of Chemistry, University of Silesia, Szkolna 9, 40-006 Katowice, Poland
email: andrzejnowok@interia.eu

N-alkoxyphenyl-hydroxynaphthalenecarboxamides are organic compounds which consist of two substituted aromatic rings (naphthalene and benzene) linked each other by CONH group. They demonstrate a strong similarity to salicylanilides – chemical compounds widely used as antibacterial, antitubercular and antimycotic agents. Biochemical experiments conducted by Gonec *et al* confirmed that N-alkoxyphenyl-hydroxynaphthalenecarboxamides show biological activity against many types of bacteria such as methicyllin-resistant MRSA [1]. Due to their ability to bond with target molecules (photosystems II) of thylakoid membranes, they also demonstrate the photosynthetic electron transport (PET) inhibition properties. Their biological activity profile depends on their chemical structure and lipophilicity properties. However, the published results don't describe the structure-activity relationship sufficiently. For that reason, we have tried to build a model which fully determines structure factors influencing the pharmacokinetic and pharmacodynamics properties of selected 38 N-alkoxyphenyl-hydroxynaphthalenecarboxamides. Our research was based on 3D-QSAR molecular modelling technique and statistical analysis PCA. The 3D-QSAR analysis revealed that the bonding strength of the analysed ligands in the active centre of photosystem II depends on the location of CONH and OH groups in naphthalene ring and also on the type and location of substituent in the benzene ring. PCA analysis showed that the structure of N-alkoxyphenyl-hydroxynaphthalenecarboxamides affects their pharmacokinetic properties, such as lipophilicity and solubility. The above-mentioned techniques revealed also that *meta*-substituted benzene ring shows optimal properties as a ligand in the active centre of photosystem II.

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P 35.

Ability of selected bacterial species to form biofilm structure on polyvinyl chloride catheters

M. Oleksy, A. Junka, M. Bartoszewicz

Medical University of Wroclaw, Faculty of Pharmacy with Division of Laboratory Diagnostics, Department of Pharmaceutical Microbiology and Parasitology, Wroclaw, Poland
email: em.oleksy@gmail.com

Background: Bacterial biofilm grow on organic tissues or such medical devices as urinary catheters, resulting in biofilm-related infections, which are extremely difficult to treat with commonly used antibiotics.
Objectives*:* The aim of our study was to assess the capacity of clinical bacterial strains to form biofilm on the surface of urinary catheters.
Material and Methods: We examined almost 400 strains from the species *Klebsiella pneumoniae*, *Escherichia coli*, *Enterobacter cloacae* and *Enterococcus faecium* (which are a common cause of urinary tract infections) for their ability to form biofilms on the surface of polystyrene microtiter plates. We used staining with the crystal violet solution and the modified Richards method. Based on the results we obtained, we chosen representative strains for biofilm testing on urinary catheters using quantitative cultures method and scanning electron microscopy.
Results: Screening tests on polystyrene plates showed that *Enterobacteriaceae* displayed higher ability to form biofilm than *E. faecium*. The results of the quantitative studies indicate significant differences between the number of living cells and the extracellular matrix of biofilm level formed on the catheters between the tested species. Especially in the case of *K. pneumoniae*, a reversed correlation was found between the number of living cells adhered to the catheter surface and the biofilm forming force on polystyrene plates.
Conclusions: The strength of biofilm formation is complex phenomenon depended from plethora of factors, including species type and surface material. The latter is a factor that obviously determines strength of biofilm structure.
ACKNOWLEDGEMENTS: Research supported with resources of the scientific project no STM.D230.16.038.

P 36.

Flavonoids – health benefits and application in human nutrition

R. Olędzki[1], K. Lutosławski[2]

[1]*Department of Biotechnology and Food Analysis, Institute of Chemistry and Food Technology, Wrocław University of Economics, Poland*
[2]*Department of Bioprocess Engineering, Institute of Chemistry and Food Technology, Wrocław University of Economics, Poland*
email: remigiusz.oledzki1@gmail.com

In recent years, in the world of science as well as in the food and pharmaceutical industry have been observed a high interest of the bioflavonoids. Flavonoids are a group of polyphenolic substances which are characterized by a high and specific biological activity. This activity creates a wide range of possibilities of using flavonoids as natural remedies in the treatment of many somatic and civilization diseases. A rational approach to nutrition should take into account the increase in the supply of flavonoids in daily diet. Therefore, the meals should be enriched by the appropriate amount of fruits and vegetables and legume seeds, which are rich in flavonoids. It is suggested that the health-promoting effect of flavonoids manifests itself, inter alia, in restoring the natural redox balance, inhibiting uncontrolled of multiplication of cancer cells and regulating of the cellular and hormonal metabolism in the human body. The results of a number of scientific studies indicate on the reverse correlation between high and regular consumption of flavonoids and lower risk of developing many diseases referred to as civilization diseases.

This report presents the characteristics of the most important sources of flavonoids and presents the influence of these antioxidants on cellular and tissue processes in the human body. This report also discusses the potential health benefits of increased consumption of foods, which are rich in antioxidants from the flavonoid group.

P 37.

Celastrol induce reactive oxygen species and apoptosis in drug resistant colon cancer cells

K. Paliszkiewicz, H. Moreira, K. Gąsiorowski

Department of Basic Medical Sciences, Faculty of Pharmacy, Wroclaw Medical University, Borowska 211 Street, 50-556 Wroclaw
email: kamila.paliszkiewicz@wp.pl and helena.moreira@umed.wroc.pl

Introduction. Celastrol a triterpene from roots of Chinese *Tripterygium wilfordii herb*, was recently described to induce cytotoxicity in several cancer cell lines via the ROS/JNK signaling pathway. In this paper we present the effect of celastrol on ROS generation and induction of apoptosis in colon cancer cells.

Methods. Doxorubicin – resistant colon cancer cells (LOVO/DX) were incubated for 1 h and 4 hrs with celastrol [0.1-20μM]. Intracellular ROS content was evaluated with DCF-DA oxidation test and mitochondrial H_2O_2 level was assessed with MitoPY1 oxidation assay. The rate of apoptotic and necrotic cells was estimated with CyFlow®Space cytometer, equipped with FlowMax software (Sysmex-Partec). after staining with AnnexinV-Alexa488 and PI.

Results. Incubation of LOVO/DX cells with celastrol at higher concentrations [5-20μM] caused significant increase of intracellular ROS content (by 21-82%), and elevated generation of mitochondrial H_2O_2 (by 26-62%), compared to the control cultures. These results correlate with the effects of celastrol on apoptosis and necrosis frequency; at the same concentrations. Addition of ROS scavenger: N-acetylcysteine (NAC) significantly lowered of both pro-apoptotic and necrotic effect of celastrol. However, lower celastrol concentrations [0.1-2.5μM] lead to slight decrease of both frequency of apoptotic/necrotic cells and ROS content.

Conclusion. Celastrol at higher concentrations quickly (in 4hrs treatment) increased apoptotic cell frequency in LOVO/DX cell cultures and the effect was strongly correlated with elevated ROS production. Increased content of mitochondrial H_2O_2 could suggest that mitochondria are an important target for celastrol action on colon cancer cells, thus celastrol-induced apoptosis would be mediated through mitochondria dependent pathways.

ACKNOWLEDGEMENTS: This work was supported by Wroclaw Medical University Grant Nr. ST.D130.16.009.

P 38.

Structure of bimodal particle multilayers

K. Pałka, P. Weroński

Jerzy Haber Institute of Catalysis and Surface Chemistry, Polish Academy of Sciences, Krakow, Poland
email: ncpalka@cyf-kr.edu.pl

The layer by layer self-assembling technique is a relatively simple method commonly used to produce multilayers with controlled bulk and surface parameters. [1] In many industrial and scientific applications, to design such materials, we have to investigate their structure. One of the common measures of the structure is the 2D pair-correlation function $g(r)$ that gives us quantitative information about local ordering of the multilayer particles. Specifically, it determines the probability density of finding a pair of projections of particle centers on the adsorption surface at a distance smaller or equal to r, normalized to unity at large distances.

In our study, we have theoretically investigated multilayers of bimodal particles of two types that have 1:2 radius ratio and opposite surface charges. We have used the extended random sequential adsorption model of hard spheres to imitate the layer by layer self-assembling process at a solid-liquid interface. We have generated five multilayers of similar thickness, each with a different single layer surface coverage in the range of 0.1 through 0.5, using self-made programs and VMD software. Then, for each multilayer, we have calculated the four 2D pair-correlation functions for small-small, small-large, large-small, and large -large particle pairs. We have also investigated variations of the correlation functions with the number of adsorbed single layers.

Our results show that the function behaviour depends on the single layer surface coverage, layer number, and particle radius ratio. We believe that our findings can be interesting and helpful for applications of multilayers in catalysis or in the formation of engineering materials with well-defined structure.

ACKNOWLEDGEMENTS: We have been supported by the EU Project POWR.03.02.00-00-I004/16. We also thank ACC Cyfronet AGH for accessing computational resources.

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P 39.

Comparative functional analysis of D-type cyclin promoters

J. Pawlonka, B. Rak, P. Wlodarski

The Department of Histology and Embryology, Laboratory of Centre for Preclinical Research, Medical University of Warsaw, Warsaw, Poland
email: janek.pawlonka@gmail.com, Brak@wum.edu.pl

The cell cycle known also as cell-division cycle is series of preparative events resulting in cell growth and division into two daughter cells. During the particular phases of the cycle (G1, S, G2, M), a cell duplicates genome, repairs DNA mutations and eventually expresses a specific set of proteins, which are necessary to conduct mitosis.

The series of these ordered and irreversible events is driven by the cyclins and their cyclin-dependent-kinases (CDKs), the specific allosteric regulatory proteins, that cause proteins phosphorylation and activation. There are four crucial cyclins (A, B, D, E), which interact with specific CDKs and trigger transitions between subsequent phases. D-type cyclins (D1, D2, D3) play essential role in the initiation of the proliferation process. Their increased expression conditions the cell transition into G1 phase, which means, that a particular cell becomes obliged to conduct cell cycle events and finally divide.

D-type cyclins genes (CCNDs) are unique among genes of other cyclins. Except the structural differences, they are also unlike others regulated by the extracellular signals, which inform whether the environment is favorable and whether there is a need for cell division, considering the whole tissue or organ. CCNDs are localized on three distinct chromosomes, their mRNA transcripts, splicing variants and posttranslational regulation often differ among each other. Their promoters share characteristic motifs through which they can be up- or downregulated, depending on transcription regulatory protein type.

Functional analysis of the promoters reveals either common or specific interactions with the main proliferative intracellular transduction pathways, but also crucial transcription factors, such as c-Myc, which interacts with D-type cyclins via E-box motif.

Our study summarizes actual knowledge about D-type cyclins promoter regulation. We consider the ways of interaction between transcription factors, DNA methylation and chromatin modifications and concludes under which circumstances aforementioned interactions lead to the net promoter activation or depression, that may play a key role in proliferation initiation. Dysregulations in these early proliferative events contribute to tumor expansion in several cancers and inappropriate tissue organization.

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P 40.

Optical and time-resolved spectroscopy for thymidylate synthase complexes characterisation

M. Prokopowicz[1,2], W. Rode[3]

[1]*Interdisciplinary Doctoral Studies in Natural Sciences and Mathematics, College of Inter-Faculty Individual Studies in Mathematics and Natural Sciences, ul. Banacha 2C, 02-097 Warsaw*
[2]*Biophysics Division, Institute of Experimental Physics, Faculty of Physics, University of Warsaw, ul. Żwirki i Wigury 93, 02-089 Warsaw*
[3]*Nencki Institute of Experimental Biology, Polish Academy of Sciences, 3 Pasteur St, 02-093 Warszawa, Poland*
email: malgorzata.prokopowicz@student.uw.edu.pl

Thymidylate synthase (TS) is an essential enzyme in the thymidylate (dTMP, one of the molecules that build DNA) salvage pathway. Due to its biological role, for over 50 years it has been recognized as a target in anticancer, antiviral and antiparasitic therapy [1-3]. The reaction catalyzed by TS involves irreversible methylation of C(5) in 2'-deoxyuridine-5'-monophosphate (dUMP), with simultaneous methyl group transfer from mTHF ($N^{5,10}$-methyltetrahydrofolate), thus leading to the formation of dTMP [4]. Between known TS inhibitors, N^4-OH-dCMP (NOH) appears particularly interesting, considering its unusual inhibition mechanism. Results of crystallographic studies with mouse TS (mTS) showed the analog to interact with the enzyme and mTHF, provoking an abortive reaction that led to formations of a binary covalent complex NOH-TS. Moreover, unlike FdUMP, NOH caused transfer of the mTHF methylene group to so far unknown destination [5, 6]. Examination of the mechanism of NOH interaction with mTS should not only benefit in clarification of this phenomenon but also enrich our knowledge of intermolecular interactions.

A novel approach is presented here, in effort to investigate of the above mentioned binary complex, involving UV absorption and fluorescence spectroscopy, with time-correlated single photon counting (TCSPC). The presence of tryptophan (Trp) and tyrosine (Tyr) (aromatic amino acids) in the secondary structure of mTS allows to employ spectroscopy. Tryptophan emission is sensitive to environmental changes, thus providing important information about *e.g.*, structural rearrangements. Application of TCSPC allows fluorescent lifetime calculation, the latter being affected by the presence of quenchers and internal factors that are dependent on the fluorophore structure. A change of mTS fluorescence lifetime helps to elucidate a fluorophore or quencher placement, as well as alteration of local environment or type of interaction [7, 8].

Our preliminary results suggest that DHF formation is present in the mTS-NOH-mTHF complexes and that the timescale of this transformations is lower than that of catalyzed reaction with dUMP.

ACKNOWLEDGEMENTS: Experiments were held in the Biophysics Division, Institute of Experimental Physics, Faculty of Physics, University of Warsaw.

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P 41.

The influence of water/powder proportion and water quality on the mechanical properties of alginate impression materials

Z. Raszewski[1], A. Nowakowska-Toporowska[2], J. Weżgowiec[3], D. Nowakowska[2]

[1]*Spofa Dental Kerr Company, Jičin, Czech Republik*
[2]*Department of Prosthodontics, Wroclaw Medical University, Wroclaw, Poland*
[3]*Department of Experimental Dentistry, Wroclaw Medical University, Wroclaw, Poland*
email: joanna.wezgowiec@gmail.com

Alginates are popular impression materials most frequently used in prosthodontics and orthodontics. However, these materials have some disadvantages, such as limited elasticity, tearing resistance, and dimensional stability.

The aim of this study was to investigate the effect of water excess or shortage and water quality on the mechanical properties of alginate impression materials. Two impression alginates, Tulip (Cavex, Holland) with color indicator, and Neocolloid (Zhermack, Italy) without this indicator, were mixed with different volumes of water, water with calcium ions, or sparkling water with CO_2. Dimensions, setting time, and hardness of the specimens were measured. Additionally, Young's modulus was calculated. The significance of differences between the mean values of different groups and the control group was assessed by Student's t-test.

The dimensional stability of both impression materials was statistically dependent on the quantity of water used for mixing. Sample storage over 24 hours of samples prepared with +15% water caused 5.00% of shrinkage for Neocolloid, and 4.41% for Tulip. The setting times of Neocolloid and Tulip were significantly prolonged when the alginates were prepared with +15% water; the addition of calcium ions shortened the setting times of both alginates. Samples mixed with the water containing Ca^{2+} ions had greater hardness and Young's modulus when compared to the alginate mixed with distilled water.

In conclusion, for mixing alginates, the manufacturers' recommended mixing ratios between powder and water should be used. To obtain the accurate setting time, hardness and elasticity, the application of distilled or demineralized water is also advised.

P 42.

The mitochondrial response of cells after electroporation in medium of high- and low- conductivity

N. Rembiałkowska, J. Kulbacka, A. Choromańska,
K. Bieżuńska-Kusiak, J. Saczko

Department of Medical Biochemistry, Wroclaw Medical University, Chalubinskiego 10 Street, 50-368 Wroclaw, Poland
email: nina.rembialkowska@gmail.com

Microsecond-duration electric pulses (electroporation method) are commonly used for research and therapeutic purposes. Electroporation (EP) can be used in combination with drugs (electrochemotherapy, ECT) to increase the uptake of chemotherapeutic drugs.

The aim of the following study was evaluation of electroporation and electrochemotherapy cytotoxicity performed by MTT assay after three difference time of incubation of the cells 24, 72 and 120 hours. We investigated human breast adenocarcinoma cells of wild type (MCF-7/WT) and doxorubicin resistant cells (MCF-7/DOX). The concentration of doxorubicin was 1.7 μM, but bleomycin were 30 nM and 300 nM. The following electroporation parameters were applied: 1000 V/cm, 100 μs, 8 pulses. As electrodes we used thin stainless-steel parallel plates (4 mm gap). In our experiments two media were used for electroporation: a high-conductive medium (SMEM, conductivity 1.53 S/m) and a low-conductive medium that contained phosphate buffer with 250 mM sucrose (STM, conductivity 0.12 S/m). The obtained results indicate that higher conductivity of the medium supports the effect of the reversible permeabilization.

ACKNOWLEDGEMENTS: This research was supported by National Science Centre, Poland, grant No.DEC-2015/19/N/NZ7/01105, (PI, N. Rembiałkowska).

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P 43.

Submicron study of the collagen fiber structure in dura mater tissue

A. Sikora[1], M. Karpińska[2], A. Kędzia[3]

[1]*Electrotechnical Institute, Division of Electrotechnology and Materials Science, M. Skłodowskiej-Curie 55/61, 50-369 Wrocław, Poland*
[2]*Active Students Association, Association of Polish Electrical Engineers SEP (Branch No 1 in Wrocław), M. Skłodowskiej-Curie 55/61, 50-369 Wrocław, Poland*
[3]*Department of Anatomy, Wrocław Medical University, T. Chałubińskiego 6a, 50-368 Wrocław, Poland*
email: a.sikora@iel.wroc.pl

The main role of dura mater, the outermost membrane of connective tissue surrounding brain and spinal cord, is to provide the mechanical protection to those organs. Failing in protection of central nervous system can be catastrophal for the condition of the subject [1]. The studies of mechanical properties of specific kinds of tissue of the CNS have been carried out in both: theoretical and experimental fashion [2]. Yet, in order to properly define the models and to understand the outcome of the measurements, one has to take into account the structure of the tissue. As dura mater contains mainly collagen fibres, the question was risen, was is its microstructural ordering in relation to its primary functionality. In order to observe the unique non-homogeneity of the bundles of collagen fibres, the atomic force microscopy (AFM) [3] was used with support of optical microscopy. It allowed to observe a unique ordering, resembling architectural structures [4] designed to provide maximum stability and mechanical performance. Obtained data extends our knowledge about the principles of the structure of the tissue and reveals new areas of research in anatomy and pathology sciences.

ACKNOWLEDGEMENTS: The authors thank the Electrotechnical Institute for Wrocław Medical University for supporting the research.

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P 44.

Preparation of recombinant vectors used for expression and purification of *Gallus gallus* Nucleobindin-2 protein and products of its proteolytic cleavage

A. Skorupska, A. Nowak, A. Ożyhar, D. Bystranowska

Department of Biochemistry, Wrocław University of Science and Technology, Wrocław, Polska
email: anna.skorupska@pwr.edu.pl and dominika.bystranowska@pwr.edu.pl

Nucleobindin-2 (Nucb2) is a Ca^{2+}/DNA binding protein. Nucb2 has been characterized as a new satiety protein in rodents. Nucb2 can be proteolytically cleaved into three peptides: nesfatin-1, nesfatin-2, nesfatin-3. Only nesfatin-1 has a significant physiological role in a food intake inhibition as demonstrated in mice and rats [1]. However, the biological significance of the remaining peptides, i.e. nesfatin-2 and nesfatin-3 still is unknown. Nucb2/nesfatin-1 is widely expressed in the brain [1] and the peripheral tissues (gastrointestinal tract [2], pancreas [3], adipose tissue [4]), which suggests involvement of this protein in variety of metabolic processes. High level of Nucb2/nesfatin-1 expression was found in breast [5], colon [6] and prostate cancer [7] and it was linked with the poor outcome and metastasis. Interestingly, Nucb2/nesfatin-1 inhibited the proliferation of ovarian epithelial carcinoma cells [8]. Additionally, Nucb2 might play a dual role in cancerogenesis. The aim of this study was to prepare four recombinant plasmid vectors (pQE-80L), containing cDNA of Nucb2, nesfatin-1, nesfatin-2 and nesfatin-3, respectively in fusion with 6×His on the N-terminus. The proper cloning process was verified by DNA sequencing. Expression analysis showed that obtained proteins are soluble, which can be further useful for its purification and biochemical and biophysical characterization of Nucb2 and products of its cleavage.

ACKNOWLEDGEMENTS: Project supported by Wroclaw Centre of Biotechnology, programme The Leading National Research Centre (KNOW) for years 2014-2018.

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P 45.

Diffusional encounter rate constants for xanthone and 2naphthoic acid. triplet-triplet energy transfer

K. Stachurska

Department of Biophysics, Institute of Experimental Physics, Faculty of Physics, University of Warsaw, Warsaw, Poland
email: karolinastach1991@gmail.com

The formation of a loose encounter complex of ligand and receptor molecules it's usually the admission to the incident many biological processes. Quantity that characterizes the rate of formation of encounter complex is encounter rate constant, which has the dimension of the ratio of the reciprocal concentration and the reciprocal of the time. There are many methods for the experimental determination of the rate constants. One of them is the nanosecond laser flash photolysis spectrometry. I will present application of this method in the research of xanthonenaphthalene-2 acid system. Conducted experimental research consist of the use of xanthone ability to act as a donor excited triplet state energy and naphthalene-2 acid, to act as an acceptor of that energy. This effect is called triplet-triplet energy transfer (TTET). The results obtained from the experiments, are subject to numerical analysis by using Dynafit, which allows to determine the rate constant of formation of encounter complex and determines mechanisms of the processes.

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P 46.

Assessment of quality of various diet patterns in European Countries

I. Staszewski, G. Różański

University of Nicolaus Copernicus in Torun Collegium Medicum in Bydgoszcz
email: igor.staszewski407@gmail.com

The good quality of consumed food in terms nutrients is important factor to prevent many diseases (cardiovascular diseases, obesity, diabetes etc.). There are many different diet patterns in various countries in europe. The aim of this study is to show results of research in which they were studied about quality of diet patterns in various european countries and pay attention to the place of Poland in a given list. To prepare this study I used PubMed base to find desirable results and choose one RCT. Results of research are expressed by five Diet-Quality Scores (DQS), Healthy Eating Index (HEI), Alternate Healthy Eating Index (AHEI), MedDietScore (MDS), PREDIMED Mediterranean Diet Score (P-MDS) and Dutch Healthy Diet Index (DHDI). Participants (n = 1480) were adults recruited from seven European Union countries. Moreover, anthropometric measures were done; Body Mass Index (BMI), Physical Activity Levels (PAL), and several biochemical markings was done too (total cholesterol, total carotenoids and omega-3 index).
Scores varied significantly beetween countries. Poland had the lower scores across all the indices. DQS higher scores were associated to lower Body Mass Indices. Better dietary patterns, were associated with markers of better nutritional status and metabolic health. This study shown us the nutritional status in Poland is not good. Actions aimed at change bad habits in polish population are needed.

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P 47.

Low-Carbohydrate diet as a treatment of Type 2 Diabetes

I. Staszewski, G. Różański

University of Nicolaus Copernicus in Torun Collegium Medicum in Bydgoszcz
email: igor.staszewski407@gmail.com

Type 2 Diabetes is currently incurable, progressive metabolic disease. Type 2 Diabetes seems lifelong therapy include special nutritional proceedings for the patient, often with use pharmacological agents. However, they are known special nutritional patterns, whoose use can decrease quantity of medicaments and gives more control over disease.

The aim of this study is to present researchs about Low-CarbohydrateDiet (LCD) as form of treatment Type 2 Diabetes. To find results of researchs We used Pubmed base, and finally worked with 3 researchs. Studies assessed glicemic control (HbA1c), weight loss, systolic blood pressure, concentration of HDL and LDL, insuline doses. The results were assessed in comparison to another special diet patterns. Attention was also paid to possible negative effects of LCD.

More results of use Low-Carbohydrate-Diet are benefical. A small amount of carbohydrate in diet could improve glicemic control (decrease of HbA1c), reduce the amount of insulin doses. Studies show us evidence for possibility of reduction the risk of Cardiovascular diseases (increase amount of High Density Lipoprotein). Use diet with small amount of carbohydrates could give negative effects include hypovitaminosis (especially vitamins B and vitamine C). However, possitive effects of diet poor in saccharides as Type 2 Diabetes treatment are not in doubt.

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P 48.

The impact of plant origin substances on the course of lactation

Z. Sycz, J. Lis-Kuberka, M. Orczyk-Pawiłowicz

Department of Chemistry and Immunochemistry, Wrocław Medical University, Bujwida 44a, 50-345 Wrocław, Poland
email: sycz.zuzanna@gmail.com

Natural substances of plant origin used by breastfeeding mothers may have a pro-lactogenic effect (galactagogues), namely initiating, maintaining and increasing milk production or anti-lactogenic (antigalactagogues) with the opposite action. Biologically active molecules of herbal medicines such as beta-glucan, trigonelline, diosgenin, phytoestrogens, thymoquinone, silymarin, silymarin-phosphatidylserine, flavonolignans, phytosterols, galegine and guanidine may have a significant impact on the course of lactation. Additionally, they improve the well-being of the mother and elicit local effects on the nipple.

So far, approximately 400 plant species are reported to have a potential impact on the course of lactation. However, experimental studies on animal models have confirmed the prolactogenic action of only a few of them: black caraway (Nigella sativa), milk thistle (Silybum marianum) and goat's rue (Galega officinalis). Clinical trials of breastfeed mothers are limited to the safest plant origin substances such as barley malt (Hordeum vulgare) and fenugreek (Trigonella foenum-graecum) and confirmed their prolactogenic activity.

Moreover, there is a lack of unambiguous results regarding the mechanism of action of individual herbs. For example, lemon balm (Melissa officinalis) gives an opposite effect depending on the dose, in low doses acting as a galactagogue, in high doses as an antigalactagogue. It is interesting that the same herbs can elicit different effects on different mothers. There are controversies regarding the action of, among others, fennel (Foeniculum vulgare) and common hops (Humulus lupulus), and herbs believed to be galactagogues for years have been recognized as antigalactagogues in recent years. However, fennel still remains a component of most lactation teas, due to the documented anti-colic effect on the breastfed baby.

P 49.

Calcium electroporation impact on mechanisms maintaining calcium homeostasis and cell structure in normal and malignant muscle cells

A. Szewczyk[1], S. Krog Frandsen[2], J. Gehl[2], M. Daczewska[1], J. Saczko[3], J. Kulbacka[3]

[1]*Department of General Zoology, University of Wroclaw, Sienkiewicza 21 St., 50-335 Wroclaw, Poland, email: anna.zielichowska@uni.wroc.pl*
[2]*Center for Experimental Drug and Gene Electrotransfer, Herlev Ringvej 75, 2730 Herlev, Denmark*
[3]*Department of Medical Biochemistry, Medical University, Chalubinskiego 10 St., 50-368 Wroclaw, Poland*
email: anna.zielichowska@gmail.com

Introduction: Electroporation mediated calcium ions is a new and very efficient anti-cancer therapy. Interestingly, an increase in intracellular calcium ions concentration induced by electroporation (EP) impacts differently on normal than malignant cells and causes disparate response of cellular components.
Materials and methods: As a study model we used normal muscle cells (C2C12 – mouse myoblast cell line) and malignant cells (RD – human rhabdomyosarcoma cell line). Each experiment was performed on undifferentiated and differentiated (D) cell lines untreated and incubated 24h after CaEP (calcium electroporation; EP, 1000V/cm; 0.5mM of [Ca^{2+}] concentration). The expression of total plasma membrane Ca^{2+} ATPase (PMCA), ryanodine receptor (RyR) and sodium-calcium exchanger (NCX) were detected. The intracellular Ca^{2+} levels in cells before, 15 min and 1 hour after treatment were measured. Cytoskeleton structure and ultrastructure were analyzed with using confocal and TEM.
Results: CaEP was significantly more efficient in RD than in normal cells. Intracellular Ca^{2+} levels after CaEP increased significantly in RD, whereas a lower increase was seen in normal cells. CaEP caused decreased expression of PMCA and NCX1 in malignant cells and RyR1 in both cell lines whereas normal cells exhibited increased expression of NCX1 after CaEP. Additionally, therapy reorganized actin cytoskeleton, inhibited zyxin expression and damaged cellular compartments such as mitochondrion, cell membrane and ER only in RD and RD-d cells.
Conclusions: These results confirmed that mechanisms maintaining calcium homeostasis are more robust in normal cells as compared to malignant cells. These studies show promising results for CaEP as an anti-cancer therapy for sarcoma.
ACKNOWLEDGEMENTS: National Science Centre, Preludium 12 (grant number 2016/23/N/NZ5/00060)

P 50.

Synthesis and ulcerogenicity study of new piroxicam derivatives

B. Szczęśniak-Sięga[1], Sz. Mogilski[2], J. Maniewska[1], B. Filipek[2]

[1]Department of Chemistry of Drugs, Faculty of Pharmacy, Wroclaw Medical University, Wroclaw, Poland
[2]Department of Pharmacodynamics, Faculty of Pharmacy, Medical College, Jagiellonian University, Krakow, Poland
e-mail: berenika.szczesniak-siega@umed.wroc.pl

Rheumatoid arthritis (RA), a chronic inflammatory multisystem disorder marked by joint pain, stiffness, swelling and multiple systemic involvements, requires a multifaceted approach for its management. It includes symptomatic treatment with analgesics as well as disease-modifying medications to alter the course of RA over time. Non-steroidal antiinflammatory drugs (NSAIDs) have been widely used for their symptomatic effects, but their use is not free from adverse effects, which may include life-threatening adverse events such as gastrointestinal (GI) bleeding and perforation. Piroxicam, an oxicam derivative which is a member of the enolic acid group of NSAIDs, is widely used in RA and osteoarthritis. Unfortunatelly, piroxicam shows increased GI toxicity, probably related to the presence of relatively long plasma half-life, thereby resulting in a more prolonged mucosal exposure [1,2]. New, safer piroxicam analogues are needed. For this purpose, a series of new piroxicam derivatives has been designed and synthesized with the modificied substituent in the thiazine nitrogen atom. In the next step new compunds were examined in a gastric ulceration test in rats. The observation using a stereoscopic microscope revealed that none of the compounds tested caused changes in the gastric mucosa. In contrast, in the same experimental conditions, piroxicam resulted in visible changes in the mucosa of the stomach. These gastrosparing effects of the studied compounds make them promising leads for new NSAIDs without gastric toxicity in the prolonged RA and osteoarthritis therapy.

ACKNOWLEDGEMENTS: This work was financed by the Ministry of Sciences and Higher Education funds for Wroclaw Medical University, project No ST-D070.17.026.

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P 51.

First digital pill in the world. The innovative way to control the pharmacotherapy of Aripiprazole in mental disorder treatment

K. Synowiec, K. Szwed, A. Tomczak

Student Science Club of Department of Toxicology, Faculty of Pharmacy, Wroclaw Medical University, Wroclaw, Poland

email: karolina.anna.szwed@gmail.com, k.synowiec92@gmail.com, aleksandrat18@wp.pl

Food and Drug Administration approved the first innovative digital pill. *Abilify MyCite* is a new solution in mental disorder therapy produced by *Otsuka Pharmaceutical and Proteus Digital Health*, which enables the control over the regularity of drug taking, for instance, when cooperation with patients, is constricted. The pill *Abilify MyCite*, beside the active ingredient – *Aripiprazolum*, contains also a sensor Ingestible Event Marker (IEM), which in the contact with gastric acid sends within 30 min. to 2 hours a signal to a receiver placed on patient's body – *MyCite Patch*. The information is sent to *MyCite APP*, an app installed on a smartphone. Aripirazol is an antipsychotic second-generation drug, which works partially agonistically on dopaminergic receptors D2 and serotoninergic receptors 5-HT_{1A}, and antagonistically on dopaminergic receptors D3 and 5-HT_{2A}. *Abilify MyCite* is used in the treatment of adults suffering from mental disorders such as schizophrenia, bipolar disorder, severe depressive disorder in combination with antidepressants. The major problem when it comes to this group of patients is the irregularity of drug taking, caused by the ineffectiveness of the therapy applied and by patient's independent decision to discontinue therapy without doctor's knowledge, which leads to the recurrence of disease symptoms. Thus, the pill *Abilify MyCite* enables both the doctor and close family to monitor the concentration of the drug in the patient's body. The majority of psychiatry specialists ensure that the use of the digital pill brings great benefits to the patients.

P 52.

Assessment of the usefulness of Nuclear Matrix Protein 22 and Bladder Cancer 4 in the diagnosis of bladder cancer

B. Szymańska[1], E. Sawicka[1], A. Długosz[1], J. Dembowski [2], M. Matuszewski[2], A. Piwowar[1]

[1]*Department of Toxicology, Faculty of Pharmacy, Wroclaw Medical University, Poland*
[2]*Department of Urology and Urological Oncology, Wroclaw University Hospital, Poland*
email: beata.szymanska@umed.wroc.pl and ewa.sawicka@umed.wroc.pl

Bladder cancer (BC) affects usually older people. Early diagnosis of BC is important because detected later has worse prognosis. Usually the cystoscopy or endoscopic bladder biopsy and cytology of urine sediment is used. This prompted researchers to look for new methods of BC diagnosis. Among the nuclear matrix proteins especially the NMP22 and BLCA-4 are of interest in bladder cancer. They are involved in genome fragmentation and cellular replication. The aim of this study was the assessment of the diagnostic value of Nuclear Matrix Protein 22 (NMP22), Bladder Cancer-4 (BLCA-4) and total pool (NMBL) in BC patients. The material (urine) was taken from 91 BC patients from the Department and Clinic of Urology and Urological Oncology at Wroclaw Medical University and from 25 healthy volunteers. The NMP22, BLCA-4 levels were measured by immunoenzymatic methods (ELISA tests). On the basis of histopathological results, the BC patients were divided into subgroups according to tumor stage (T) and grade (G). The statistical analysis was performed using STATISTICA 12. The levels of NMP22, BLCA-4, NMBL ($p<0,001$) were significantly higher in BC patients than in the control group. In the subgroups with different tumor stage (T) and tumor grade (G) differences were noted for BLCA-4 and NMBL. Study indicates the higher diagnostic value of the total protein pool of the nuclear matrix (NMBL) than the single protein, NMP22 or BLCA-4, in bladder cancer. The project received the permission of the Bioethics Committee of Wroclaw Medical University.

P 53.

Influence of cistus and pomegranate extracts on ROS generation in V79 cells

A. Ślęzak[1], H. Moreira[2], B. Wiatrak[2], J. Oszmiański[3], K. Gąsiorowski[2]

[1]*Department of Paediatric Bone Marrow Transplantation, Oncology and Hematology, Wroclaw Medical University, Wroclaw, Poland*
[2]*Department of Basic Medical Sciences, Wroclaw Medical University, Wroclaw, Poland*
[3]*Department of Fruit, Vegetable and Cereals Technology, Wroclaw University of Environmental and Life Sciences, Wroclaw, Poland*
email: kazimierz.gasiorowski@umed.wroc.pl

Objective: We compared an impact of the water extracts obtained from cistus herb (*Cistus incanus* L.) and pomegranate peel (*Punica granatum* L.) on ROS and on H_2O_2 content within V79 cells (Chinese hamster pulmonary fibroblasts). after short (1h) and long (48hrs) exposure time.
Methods: Intracellular ROS level was estimated with DCF-DA oxidation assay, and mitochondrial H_2O_2 content was assessed with the MitoPy oxidation method. Samples were analyzed with CyFlow® Space cytometer. The results were correlated with the Pearson method, for low [0.1-5μg/ml] and high [10-100μg/ml] concentrations of the extracts.
Results: The extracts added for 1 h at high concentrations to V79 cultures induced ROS and H_2O_2 production, however 48-hour exposition lead to significant decrease in the amount of generated free radicals. Strong positive correlation between results of DCF-DA and MitoPY was calculated for both cistus and pomegranate extracts at high concentrations assays in the tests after 1h. Differently, at low concentrations after 1-hour incubation of cells with the extracts a strong negative correlation between DCF-DA and MitoPY assays was calculated in the case of both extracts whereas after 48 hrs of incubation a positive correlation with moderate strength for cistus extract and negative correlation with moderate strength for pomegranate.
Conclusions: The extracts exhibited antioxidant activity at low concentrations while a pro-oxidant effect at high concentrations. The pro-oxidant effects were significantly decreased after prolonged incubation with the extracts. This is probably the result of the activation of intracellular antioxidant mechanisms, which need a longer time to reveal their full expression.

P 54.

β-Lactoglobulin as a platform for designing biologically active carriers: simulation and experiment

G. Turner[1,2], S. Świątek[1], P. Komorek[1], B. Jachimska[1]

[1]*Institute of Catalysis and Surface Chemistry, PAS, Cracow, Poland*
[2]*Department of Chemical and Process Engineering, University of Strathclyde, Glasgow, United Kingdom*
email: sylwia.anna.swiatek@gmail.com, paulina-komorek@wp.pl, ncjachim@cyf-kr.edu.pl

This study reports the effectiveness of β-lactoglobulin (LGB)as a potential drug carrier for the anaesthetic tetracaine (TCA) under varying environmental conditions (pH, ionic strength, concentration, LGB-TCA complex molar ratio). TCA is a linear, hydrophobic molecule of length ~1.5mm, similar to the natural ligands (fatty acids) of LGB. In this work, spectroscopic UV-vis and Laser Doppler Velocimetry (LDV) were utilised to determine the physicochemical properties of LGB and LGB-TCA complex in a sodium chloride solution. Electrophoretic mobility measurements showed that in comparison to a pure LGB solution, the addition of TCA deceased the zeta potential as well as shifting the isoelectric point to a greater pH. This suggests the formation of LGB-TCA complexes. UV-vis spectra for LGB, TCA and its complex were recorded. The binding constant (KUV) of the complex was calculated. The adsorption capacity of LGB on the gold surface was studied using quartz crystal microbalance with dissipation energy monitoring technique (QCM-D). From this technique, the optimal conditions (pH, ionic strength, concentration) to form the LGB-TCA complex were determined. Molecular docking was implemented to predict the predominate binding mode of the LGB-TCA complex in the software package SCIGRESS, version FJ 2.8 (a multiplatform molecular design, modelling and dynamics software suite). The molecular docking software simulations produced and ranked a range of poses using a genetic algorithmand the knowledge-based scoring function (PMF04). From these poses, the potential conformation of the complex can be visualised, and the surroundingamino acid groups analysed; comparison can be made with other docking and x-ray crystallography conformationsfrom literature.

ACKNOWLEDGEMENTS: This work was supported by Grant NCN OPUS 2016/23/B/ST5/02788 and Erasmus+No.2017-1-UK01-KA103-036473.

P 55.

Photodynamic therapy – effects on VEGF, MIF and IL-10 secretion by colon cancer cells in vitro under aerobic and anaerobic conditions

A. Kawczyk-Krupka[1], W. Latos[1], Z. Czuba[3], K. Sieroń-Stołtny[1], G. Cieślar[1], K. Wasilewska[4], M. Potempa[2], W. Król[3], A. Sieroń[1]

[1]Clinical Department of Internal Diseases, Angiology and Physical Medicine, Center for Laser Diagnostics and Therapy, Medical University of Silesia, Bytom, Poland
[2]Medical University of Silesia, Zabrze, Poland
[3]Department of Microbiology and Immunology, Medical University of Silesia, Zabrze, Poland
[4]School of Clinical Medicine, University of Cambridge, United Kingdom
email: kat.wasilewska@gmail.com

Cancer therapy is often based on a combination of conventional methods of cancer treatment and immunotherapy. Photodynamic therapy (PDT) is one of the immunomodulating methods used in oncology. We examined how PDT influences the secretory activity of colon cancer cells in vitro, especially the secretion of vascular endothelial growth factor (VEGF), macrophage migration inhibitory factor (MIF) and interleukin 10 (IL-10), in aerobic and anaerobic conditions.

P 56.

The influence of extraction methodology on the chemical composition and properties of propolis from different regions of Poland

J. Wężgowiec[1], A. Wieczyńska[2], J. Kulbacka[3], D. Nowakowska[4], W. Więckiewicz[4]

[1]Department of Experimental Dentistry, Wroclaw Medical University, Wroclaw, Poland
[2]Department of Organic and Pharmaceutical Technology, Faculty of Chemistry, Wroclaw University of Science and Technology, Wroclaw, Poland
[3]Department of Medical Biochemistry, Wroclaw Medical University, Wroclaw, Poland
[4]Department of Prosthodontics, Wroclaw Medical University, Wroclaw, Poland
email: joanna.wezgowiec@umed.wroc.pl

In modern medicine, the interest in natural products is constantly growing. Propolis – a mixture of materials collected by bees – is one of the most intensively investigated substance with a variety of biological properties.

The aim of the presented study was to determine how the extraction methodology influences the chemical composition and antioxidant properties of propolis collected in different regions of Poland.

Propolis preparations were achieved by means of extraction with ethanol, hexan or multistage extraction with ethanol/hexan or hexan/ethanol. The total polyphenolic content was analyzed colorimetrically using the Folin-Ciocalteu method and the total flavonoid content - using the aluminium chloride method. 2,2-diphenyl-1-picrylhydrazil (DPPH) radical scavenging assay was performed to determine the antioxidant activity of propolis extracts.

The presented results demonstrated differences in the chemical composition and antioxidant activity of different propolis products, depending on the extraction technique. Extraction with ethanol, both proceeded by the extraction with hexan or without it, was the most effective method of obtaining active substances from the studied Polish propolis.

ACKNOWLEDGEMENTS: The authors would like to thank Prof. K.A. Wilk and Prof. R. Gancarz from Department of Organic and Pharmaceutical Technology at Faculty of Chemistry of Wroclaw University of Science and Technology for their valuable comments and providing access to laboratory rooms and equipment.

This work was financed by Wroclaw Medical University – grant no. STM.B022.17.013 (J. Wężgowiec) and by the statutory activity subsidy for the Faculty of Chemistry of Wroclaw University of Science and Technology.

P 57

Influence of ligands on gold nanoclusters' photoluminescence

A. Więcek, M. Waszkielewicz, M. Samoć, J. Olesiak-Bańska

Advanced Materials Engineering and Modelling Group, Wroclaw University of Science and Technology, Wybrzeze Wyspianskiego 27, 50-370 Wroclaw, Polande
Email: wiecek.a95@gmail.com and joanna.olesiak-banska@pwr.edu.pl

Objective: Examining the influence of various ligands on small-sized gold nanoclusters properties. Furthermore, the effect of solvents on photoluminescence intensity was analyzed.
Materials and methods: Syntheses of ATT-Au (ATT – 6-aza-2-thiothymine), ATT/Arg-Au (Arg – L-arginine), $Au_{18}SG_{14}$ and AuAgGSH (SG – glutathione) nanoclusters were carried out. The photoluminescence quantum yield was investigated using UV-Vis absorption spectroscopy and photoluminescence (PL) spectroscopy. PL quantum yield values were calculated by comparing the synthesized materials with well-known references (Rhodamine B, Rhodamine 6G and Coumarin 153). Finally, the gold nanoclusters photoluminescence intensity was analyzed in different mixtures of solvents: water, ethanol, ethanol + water, water + PAH (polyallylamine hydrochloride).
Results & conclusions: Studies show that the type of ligands, the gold core composition and the type of a solvent affect PL quantum yield (QY) values. [1], [2] The highest QY values were observed in AuAgGSH nanoclusters (about 16%). Interestingly, analogous $Au_{18}SG_{14}$ nanoclusters reveal decreased quantum yield values (6%). It has been proved an enhancement of ATT-Au photoluminescence by adding L-arginine into gold nanoclusters capping layer can be achieved. The fluorescence enhancement mechanism depends on reducing intramolecular vibration and rotation of ATT groups via interactions between ATT and L-arginine groups. It leads to a significant reduction of energy loss channels and increase of PL QY values. It is believed that highly luminescent, possessing low toxicity and photostable gold nanoclusters can be used in biomedical imaging [3] and clinical diagnosis.
ACKNOWLEDGEMENTS: The research were carried out within the First Team NONA project of the Foundation for Polish Science co-financed by the European Union under the European Regional Development Fund.
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P 58.

The importance of F domains in nuclear receptors proper functioning

A. Więch

Department of Biochemistry, Faculty of Chemistry, Wrocław University of Science and Technology, Wrocław, Poland
email: anna.wiech@pwr.edu.pl

Progesterone – responsible for pregnancy maintaining, estrogen – crucial during puberty and in lipid and calcium metabolism, testosterone – important in sex formation and protein synthesis are steroid hormones. Calcitriol, the active form of vitamin D, regulates calcium-phosphate metabolism and maintains proper skeleton integrity. Its role is also known in cell proliferation processes and in the action of the immune system. Aforementioned molecules are ligands for specific transcription factors – nuclear receptors (NRs) [1].
In the structure of NRs five domains may be distinguished: non-conserved N-terminal domain (NTD) with one constitutive transactivation region (AF-1), DNA binding domain (DBD) with two zinc-fingers, elastic hinge region that very often contains nuclear localization signal (NLS), ligand binding domain (LBD) with the second transactivation site (AF-2) and F domain at the C-terminus [2]. Binding ligand molecule by NR enables the expression of genes that possess special response elements in their promoter sequence. Chromatin decondensation occurs allowing RNA polymerase to bind [3].
Among mammals F domain is hypervariable in terms of sequence, length, function or even existence, however for progesterone receptor (PR) its ability to control transcription activation by recruiting co-repressor or co-activator molecules was stated. F domain stabilizes the conformation of LBD and controls subcellular localization in estrogen related receptor (ERR) and takes part in dimerization of androgen receptor (AR). The affinity and transcription activity of vitamin D receptor (VDR) may be also modulated by its F domain [4]. Thus, exploring the subject of F domain is pivotal in full understanding the NRs' mechanism of action.

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P 59.

Interactions of human serum albumin with cationic surfactants: a spectroscopy and molecular modelling

P. Wójcik, T. Janek, Ż. Czyżnikowska

Department of Inorganic Chemistry, Wroclaw Medical University, Wroclaw, Poland
email: tomasz.janek@umed.wroc.pl and zaneta.czyznikowska@gmail.com

The binding of several different categories of small molecules to human serum albumin (HSA) has been studied for many years through different spectroscopic techniques to elucidate details of the protein structure and binding mechanism. In this work results are presented for the interaction of HSA with several cationic surfactants, namely, (2-dodecanoyloxyethyl) trimethylammonium bromide (DMM-11), (2-dodecanoyloxypropyl) trimethylammonium bromide (DMPM-11), and (2-pentadecanoyloxymethyl) trimethylammonium bromide (DMGM-14) as monitored by fluorescence spectroscopy of intrinsic tryptophans and circular dichroism spectroscopy. Furthermore, the size of the micellar aggregates of cationic surfactants were studied by dynamic light scattering technique (DLS). The hydrodynamic radii, micellar volumes and aggregation numbers were calculated using a method based on density functional theory (DFT). The binding sites of the molecular systems in HSA have been located with the aid of docking studies.

The fluorescence intensity of HSA has changed as the concentration of cationic surfactants increased and this effect was attributed to the formation of surfactant-HSA complexes. Finally, CD and DLS results revealed the occurrence of changes in the secondary structure of the protein in the presence of cationic surfactants. The molecular docking results indicated that cationic surfactants bind with HSA at hydrophobic pocket domain with hydrogen bond interactions.

ACKNOWLEDGEMENTS: The authors would like to kindly thank Dr. Jacek Łuczyński for the opportunity to carry out research on the substances synthesized by him.

P 60.

The role of interaction of estrogens with xenoestrogens in hormone-dependent cancers in women

W. Wójcik[1], M. Wolniak[1], K. Zdybel[1], E. Sawicka[2], A. Piwowar[2]

[1]*Student Scientific Club of Department of Toxicology, Faculty of Pharmacy, Wroclaw Medical University, Wroclaw, Poland*
[2]*Department of Toxicology, Faculty of Pharmacy, Wroclaw Medical University, Wroclaw, Poland*
email: wiktoriabwojcik@gmail.com

Xenoestrogens are exogenous chemical compounds that can bind to estrogen receptors (ERα, ERβ). They can mimic the functions of endogenous hormones, acting as their agonists or antagonists. There are synthetic or natural substances including metals (Cr, Cd, Cu, Ni, Pb) synthetic compounds, ingredients of plastics, as well as natural plant substances referred to as phytoestrogens. Due to the increasing prevalence of xenoestrogens in the environment and the constantly increasing number of cases of hormone-dependent cancers in recent years, we can draw a conclusion about interdependence between them. Although the problem of the effect of xenoestrogens on hormone activity is still little known, the results of numerous studies suggest the importance of this problem. It should be remembered that estrogens are compounds with high biological activity, so hormonal imbalance can have serious health effects, including cancers. It is noteworthy that xenoestrogens may accumulate in the body throughout life, increasing the risk of cancers derived from estrogen-dependent tissues, i.e. breast, ovarian and endometrial cancers. Epidemiological research suggests that in approximately 90% of cases, environmental factors are responsible for the development of this type of cancer and to a lesser extent – genetic factors. Considering this dependence, additional stimulation by xenoestrogen substances may theoretically increase the risk of cancer and also accelerate the development of this disease. Numerous sources, long-term time of exposure and the possibility of xenoestrogens accumulation, may affect the significant contribution of these compounds to the initiation of pathological processes, such as impaired fertility or cancer in women.

P 61.

Intrinsically disordered proteins and their short linear motifs

K. Wycisk

Department of Biochemistry, Wroclaw University of Science and Technology, Wroclaw, Poland
email: krzysztof.wycisk@pwr.edu.pl

There are two theories explaining interactions between proteins and substrates – the lock and key theory [1] and the induced fit theory [2]. Both theories assume that at physiological conditions protein molecule have just one, stable three-dimensional structure. Now we know that such assumption is large simplification and ignores the fact that some proteins exist and function in disordered state. These so called intrinsically disordered proteins (IDPs) are a class of proteins that do not have or have only partial secondary structure and completely lack tertiary structure in native conditions. However, after binding their ligands some IDPs can adopt stable conformation [3]. Disordered proteins or disordered regions of proteins commonly interact via characteristic short stretches of sequence known as short linear motifs (SLiMs). These motifs are functional modules, usually with a length of 3-10 amino acids [4]. In general, SLiMs mediate interactions with globular domains, but they can also create signals like targeting signals or degradation motifs. Insertion or deletion of sequences containing SLiMs in form of alternative exons, strongly influence functionality of isoforms [4]. Therefore, mutations in SLiMs often lead to diseases, e.g. cancer [5]. What is also important, different pathogens can mimic host SLiMs and influence crucial processes in host organism [5]. Fortunately, growing knowledge about SLiMs provides background for developing effective strategies to neutralize pathological conditions and even to develop drugs mimicking ligand SLiMs [5].

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P 62.

Ferroptosis – a new, iron-dependent form of cell death by lipid peroxidation

P. Zuziak, M. Kotas, M. Tyliszczak

Department of Medical Biochemistry, Wroclaw Medical University, Chałubińskiego 10, 50-368 Wroclaw, Poland
email: pat.zuziak@gmail.com and kotasmarek@interia.pl

Ferroptosis is a recently (2012) discovered form of iron-dependent cell death that significantly differs from the other known types of cell death, such as necrosis or apoptosis. It is caused by peroxidation of lipids in the cell membrane, which is caused either by inhibition of lipid-repairing enzyme glutathione peroxidase (GPx4), or by the depletion of intracellular glutathione reserves. This can be caused by various mechanisms, many of which have been discovered in recent years. Amongst others, the inhibition of cystine uptake (system Xc inhibition), or inhibition of the transsulfuration pathway leads to the depletion of intracellular glutathione. Recent studies have shed more light on the molecular mechanisms of ferroptosis, and many more ferroptosis-inducing agents were discovered. Moreover, other studies have shown that ferroptotic cell death plays a role in already known physiological and pathophysiological processes in the human body. However, presumably the most promising findings, were made in the field of oncology – many neoplastic cell lines were found to be vulnerable to ferroptosis-inducing factors.

The purpose of this paper is the review of advances in the field of ferroptosis, and description of its known molecular mechanisms. We will take a look on ferroptosis-inducing factors, the differences between ferroptosis and other types of cell death and – finally – its potential applications in the treatment of neoplasms, especially particularly resistant tumours.

P 63.

Features of the biochemical status of blood in women with the threat of miscarriage

A. Lemesh[1], D. Valchkevich[1], A. Valchkevich[2]

[1]*Department of Normal Anatomy, Grodno Medical State University, Grodno, Belarus*
[2]*Department of Clinical Laboratory Diagnostics and Immunology, Grodno Medical State University, Grodno, Belarus*

An unfavourable demographic situation is one of the most important social problems. Therefore, increasing fertility and reducing reproductive losses are the priorities of modern reproductive medicine.
Aim: to reveal the correlation between the parameters of the biochemical status of blood in various terms of the pregnancy at threat of miscarriage.
Materials and methods: 68 conclusions of the biochemical analysis of blood in women with threat of miscarriage were investigated in terms of 16-36 weeks.
Results: relationship between AlAT and AsAT ($R=0.88$, $p<0.05$) was established in women up to 20 weeks gestation.
Women with gestational age from 20 to 30 weeks had following relationships: total protein with bilirubin ($R=0.4$, $p<0.05$) and AsAT ($R=0.55$, $p<0.05$), creatinine and urea ($R=0.72$, $p<0.05$), bilirubin with AsAT ($R=0.5$, $p<0.55$) and AlAT ($R=0.5$, $p<0.05$), AsAT and AlAT ($R= 0.7$, $P<0.05$).
The relationship between the total protein and AsAT ($R=0.5$, $p<0.05$), urea and creatinine ($R=0.73$, $p<0.05$), bilirubin with AsAT ($R=0.6$, $p<0.05$) and AlAT ($R=0.8$, $p<0.05$), AsAT and AlAT ($R=0.7$, $p<0.05$) was established in term after 30 weeks.
Conclusion: women with a threat of miscarriage had a high positive correlation in biochemical analysis between the six most commonly identified and informative laboratory indicators. At the time of pregnancy with the threat of interruption to 20 weeks, the highest correlations are noted between transaminases of the blood. After 30 weeks, it is necessary to pay attention to the "liver diagnostic panel", since there is a high correlation between bilirubin and AlAT, which is an organ-specific enzyme of the liver.

P 64.

Insulin amyloid spherulites and factors influencing their formation – spectroscopic and microscopic study

A. Grzesik, K. Brach, J. Olesiak-Bańska, M. Samoć

Faculty of Chemistry, Advanced Materials Engineering and Modelling Group, Wroclaw University of Science and Technology, Wroclaw, Poland
email: joanna.olesiak-banska@pwr.edu.p

Protein misfolding and aggregation are common physical processes which have been associated with neurodegeneration leading to various diseases such as Alzheimer's, Parkinson's or Huntington's [1]. Amyloid fibril, exhibiting a cross-b sheet quaternary structure, is probably one of the most well-known examples of misfolded and aggregated proteins. Depending on the aggregation mechanism and experimental conditions, different types of amyloid aggregate structures may be observed [2]. Amongst these morphologies, amyloid spherulites are of high interest. The spherulites are built of amyloid fibrils growing radially from a protein core, which does not exhibit amyloid structure [3]. They are also found in vivo in Creutzfeldt-Jakob disease [4].

The aim of this work was to investigate the properties of amyloid spherulites obtained from bovine insulin under different experimental conditions. Depending on the pH values, salt presence and its concentration, formation of spherulites was inhibited and the size of aggregates was changing. UV-Vis and fluorescence spectroscopy, AFM and one-photon polarized light microscopy were applied to characterize obtained structures. Additionally, taking into account nonlinear properties of amyloid fibrils [5, 6], two-photon microscopy was used to imagine the spherulites without using any probes or labels.

ACKNOWLEDGEMENTS: *The research were carried out within the First Team NONA project of the Foundation for Polish Science co-financed by the European Union under the European Regional Development Fund.*

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