

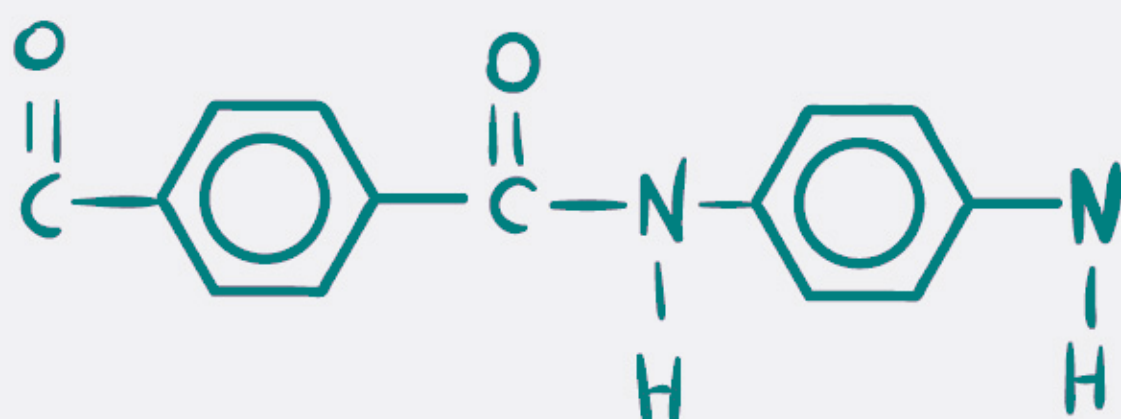
4th International Cholnoky Symposium

Abstract book



10-11 May, 2018

Dean's Conference Room,
University of Pécs Medical School,
12 Szigeti Street, Pécs





EMBERI ERŐFORRÁSOK
MINISZTERIUMA



EMBERI ERŐFORRÁS
TÁMOGATÁSKEZELŐ





Organizers:

Dr. Krisztian Kvell

Petra Matyas

Dr. Timea Bencsik

Dr. Miklos Poor

Kitti Garai

Krisztina Banfai

Bettina Tigyi

Zsófia Gampel

Krisztina Ferenczi



4th International Cholnoky Symposium

10-11 May, 2018

Program

THURSDAY, 10 MAY 2018

11:00-11:50 Registration/Information Desk

11:50-12:00 Symposium opening

12:00-14:30: Pharmaceutical Sciences Session
Keynote Speaker: **Dr. Mary Keen**

14:30-15:00 Coffee Break

15:00-17:30 Biotechnology and Health Sciences Session
Keynote Speaker: **Dr. Károly Tihanyi**

19:00- Dinner at Kalamáris Restaurant*

FRIDAY, 11 MAY 2018

8:00-9:00 Registration/Information Desk

8:00-10:00 Medical Sciences Session I.
Keynote Speaker: **Dr. Tamás Fülöp**

10:00-10:30 Coffee Break

10:30-12:30 Medical Sciences Session II.

12:30-12:40 Symposium closing

***Registration required**

Duration of presentation: 20+5 minutes for keynote speakers and 10 minutes for junior lecturers



4th International Cholnoky Symposium

10-11 May, 2018

Program

THURSDAY, 10 MAY 2018

11:00-11:50 Registration/Information Desk

11:50-12:00 Symposium opening

12:00-14:30 Pharmaceutical Sciences Session

Keynote Speaker:

Dr. Mary Keen: Problems with the safety testing of biopharmaceuticals.

1. **Anett Szabo:** Absorption of the ibuprofen and the appearance of its oxydative metabolits from small intestinal perfusate and the bile.
2. **Dr. David Sinka:** Evaluation of modified-release formulation containing Silybum marianum herb extract for oral application.
3. **Dr. Violetta Mohos:** Effects of quercetin, chrysin, and their metabolites on the xanthine oxidase-catalyzed biotransformation of 6-mercaptopurine.
4. **Adrienn Horvath:** Effects of different parameters of herbal tea preparation on active compounds of Silybi mariani fructus, Uvae ursi folium, and Rosae pseudofructus.
5. **Fatemeh Kenari:** Study on interaction of reduced glutathione (GSH) with some Chalcone analogues.
6. **Virag Voros:** Interactions of mycotoxin metabolites 2'R-ochratoxin A and dihydrocitrinone with human serum albumin.
7. **Dr. Eszter Fliszar-Nyul:** Analysis of capsaicin and dihydrocapsaicin metabolism of the small intestine in the diabetic rat by HPLC-FLD.
8. **Bettina Mariann Tigyi:** Chemical analysis of essential oils using gas chromatography and their antibacterial activity with TLC-Direct Bioautography.
9. **Krisztina Ferenczi:** Optimization of determination of total polyphenol content in *Origanum vulgare* L.
10. **Dr. Andras Fittler:** Patient experience and attitude toward medicines and dietary supplements purchased outside the traditional supply chain.
11. **Barna Kollar:** Steroid-harboring nanoparticles provide anti-inflammatory response with less adverse effect.

***Registration required**



14:30-15:00 Coffee Break

15:00-17:30 Biotechnology and Health Sciences Session

Keynote Speaker:

Dr. Karoly Tihanyi: Biotechnology vs. small compound drug research. Advantages and limitations.

1. **Dr. Zelma Faisal:** Removal of zearalenone and its metabolites from aqueous solutions by insoluble β -cyclodextrin bead polymer.
2. **Vivien Fejes:** The Effect of Ozone on Forensic Samples.
3. **Ferenc Boda:** Effect of Vipera ammodytes ammodytes Snake Venom on the Human Cytokine Network.
4. **Krisztina Banfai:** Significance of Wnt4-exosomes in thymic senescence.
5. **Szabina Hodovan:** Investigating the everyday life of people with Post-Polio Syndrome.
6. **Kitti Garai:** Exercise alleviates senescence-related parameters in healthy, young individuals.
7. **David Bodo:** Muscle activation and movement pattern in bodyweight exercises.
8. **Vivien Laski:** The performance improving effect of swimming in synchronized swimming.
9. **Zoltan Adam:** Physical activity as the saviour of circulation, data of a lifestyle intervention program.
10. **Judit Magyar:** The effects of non-caloric artificial sweetener consumption on Microbiom.
11. **Zsofia Rita Balint:** The frequency of muscle dysmorphia and eating disorders and the examination of nutrition among bodybuilders.

19:00- Dinner at Kalamáris Restaurant*

***Registration required**

Maximum time of the presentation is 25 minutes + 5 minutes discussion for keynote speakers and 10 minutes + one question for all lecturers.



FRIDAY, 11 MAY 2018

8:00-9:00 Registration/Information Desk

8:00-10:00 Medical Sciences Session I.

Keynote Speaker:

Dr. Tamas Fulop: Immunosenescence and inflammaging: an intricate connection.

1. **Jonatan Pasitka:** Examination of biologically relevant electron transfer in fotolyases with ultrafast laser spectroscopy.
2. **Dr. Benedek Tinusz:** Histological and Incidence Trends of Esophageal Cancer in a 26-year Period.
3. **Antonio Barać:** Hematopoiesis requires a self-generating and well-organized process of differentiation of hematopoietic stem cells to maintain cell life span and their diversity.
4. **Ivana Gašpar:** Ferokines – Diagnostic value of laboratory tests.
5. **Henrietta Papp:** Current trends in Zika virus research.
6. **Vlatka Stojčić:** The role of the gut microbiota in etiology of mental disorders.
7. **Husseiny Mohamed Abdelwahab:** Role of mitochondria in LAM.

10:00-10:30 Coffee Break

10:30-12:30 Medical Sciences Session II.

1. **Diana Derdak:** Toward understanding the binding of a polyene antifungal drug to serum albumins.
2. **Orsolya Homoki:** Could irradiation be more efficient on breast cancer cell-line?
3. **Dr. Katalin Nora Lorincz:** Epilepsy- Surgery?
4. **Barbara Brandt:** Analysis of temozolomide resistance in glioblastoma cell lines.
5. **Lina Hudhud:** Extracellular vesicles and Wnt signaling in the background of Chronic Obstructive Pulmonary Disease.
6. **Bence Kiss:** Examination of BFSP1 (Beaded Filament Structural Protein 1 or Filensin) tumor specific splice variants in ex vivo human tumorous tissue and human serum samples.
7. **Dora Buzas:** Zika virus replication in human blood platelets.

12:30-12:40 Symposium closing

***Registration required**

Abstracts

Role of mitochondria in Lymphangiolyomatosis

Elhusseiny Mohammed Abdelwahab, Judit Rapp, Diana Feller, Luca Jaromi, Peter Bai, V
Krimskaya, Krisztian Kvell, Judit E. Pongracz

Department of Pharmaceutical Biotechnology, University of Pécs

Introduction: Lymphangiolyomatosis (LAM) is rare deadly disease affecting women in fertile age. Symptoms are similar to asthma, COPD and lung infections making LAM widely misdiagnosed^{1,2}. Two types of LAMs have been identified, the inherited form that is caused by a TSC gene mutation (TSC-LAM) and the acquired form that is mostly linked with Tuberous Sclerosis (sporadic-LAM) ¹. Treatment options are limited to controlling the growth of the affected smooth muscle cells using mTOR inhibitor drugs, or lung transplantation in advanced cases¹. Mitochondria are the powerhouses of cells and control a machinery for cell metabolism³. For this they are promising therapeutic targets in different diseases and cancers^{3,4}. In this case too, mitochondria can be of key importance in LAM treatment.

Aims: In the present study, we investigated the possibility that mitochondrial dysfunction is actively involved in LAM development and plays an important role in cyst formation and progression of the disease.

Materials and Methods: After culturing, the six cell lines (2 smooth muscle cells and 4 LAM cells), sections preparation for electron microscopy, Rhodamine 123 for flow-cytometry, RNA isolation was performed (Macherey-Nagel, Germany) following the reverse transcription, quantitative RT-PCR and SeaHorse XF96.

Results and discussion: Electron microscope analysis revealed that mitochondria in LAM cells are smaller and darker than those in normal SMCs referring to reduced function. Rhodamine 123, sequestered by active mitochondria, has been used to confirm inferior mitochondrial function supported by SeaHorse XF96 metabolic measurements. Upregulation of several mitochondrial genes was detected by RT-PCR.

Conclusion: Alterations of mitochondrial function in LAM cells (both morphological and molecular) suggest that mitochondria are promising therapeutic targets of LAM disease treatment.

Physical activity as the saviour of circulation, data of a lifestyle intervention program

Zoltan Adam, Kitti Garai, Krisztian Kvell, Adam Gyebrovski, Marta Wilhelm

Institute of Sport Sciences and Physical Education, Faculty of Sciences, University of Pécs

Background: Exercise has long been identified as an important factor of health maintenance in individuals of all ages. A suggested amount of regular daily physical activity can lower risk factors of all-cause mortality and has a leading role in primary prevention of cardiovascular disease per se by keeping the circulatory system in good condition.

Aim: To prove that regular moderate intensity exercise can result in significant changes in many health parameters a lifestyle intervention program was organised. 20 university students participated, 16 of them ($23,85 \pm 3,15$ years, 2 males, 14 females) completed the full intervention.

Methods: The six-month long project built up of 1 hour of resistance and aerobic training three times a week. Three major health parameter measurements were done to gather anthropometric (skinfolds, circumferences), cardiorespiratory (blood pressure, heartrate), blood test and physical endurance (6 Minute Walking Test) data. A one-week long activity monitoring was performed to measure possible behavioural adaptation to the training.

Results: Many of the anthropometric parameters decreased while the clear muscle mass increased ($p \leq 0,05$). The time spent in sedentary state decreased, while time spent in light to moderate intensity raised ($p \leq 0,05$). Both systolic and diastolic pressures reduced even though only systolic pressure changes proved to be significant ($p \leq 0,05$). A decrease in resting- and a slight escalation of recovery HR is visible during the intervention ($p \leq 0,05$). There were significant improvements in the blood components also.

Conclusion: 6 months of moderate intensity mixed exercise training is associated with the improvement of overall fitness level even in young healthy university students with normal range values.

Keywords: lifestyle intervention, regular physical activity, cardiovascular fitness

The frequency of muscle dysmorphia and eating disorders and the examination of nutrition among bodybuilders

Zsófia Rita Balint, Eva Polyak

Institute of Nutritional Science and Dietetics, Faculty of Health Sciences, University of Pécs

Introduction: The purpose of this study was to determine the presence of muscle dysmorphia, exercise dependence and eating disorders in Hungarian bodybuilders and to examine the relationship between these disorders especially by looking at their protein intake.

Material and method: For the research we used self-edited and standard questionnaires such as EAT-26, EDS, MDDI, BIG-O and OLEF 24 hours dietary diary. Anthropometric measures had been done by OMRON BF 511 body composition measurer. The result of the dietary diary has been analyzed by NutriComp Étrend 4.0 software and all of the results have been analyzed by SPSS version 22. We used Odds ratio, Spearman's correlation, two pattern independent t-test, one pattern t-test, Mann-Whitney test and Kruskal-Wallis test for analyzing. The results were significant if $p \leq 0,05$.

Results: Based on EAT-26, from 67 person 11 person showed risk for eating disorder. 9 person showed the risk of exercise dependence based on EDS. About muscle dysmorphia MDDI results showed that 10 person and BIG-O results showed that 59 person have the risk of this disorder.

Subjects who had muscle dysmorphia by MDDI had a bigger chance to have some kind of eating disorder too (OR=4,762 95% CI:1,071-21,172). Those who had muscle dysmorphia based on BIG-O had a bigger protein intake ($p=0,046$) than those who got in the „non dysmorphic” group.

Conclusion: The result of this study that muscle dysmorphia, eating disorders and exercise dependence appear in the Hungarian bodybuilding population and often these disorders turn up next to each other.

Keywords: muscle dysmorphia, eating disorder, exercise dependence, high protein intake

Significance of Wnt4-exosomes in thymic senescence

Krisztina Banfai, Kitti Garai, David Ernszt, Judit E. Pongracz, Krisztian Kvell

Department of Pharmaceutical Biotechnology, Faculty of Pharmacy, University of Pécs

It is well-known, that Wnt4 plays an essential role during the development of the thymus via regulating thymopoiesis and maintaining the cellular composition of TECs (thymic epithelial cells). When thymic senescence starts Wnt4 is down-regulated, while PPAR γ is up-regulated and triggers adipose involution. Of note, miR27b was described to suppress PPAR γ . Both the Wnts and various miRNA species have been reported to reside in exosomes in other contexts. Our aim was to confirm the presence of Wnt4 and miR27b in TEC exosomes, detect their effect and follow their migration both in vitro and in vivo. Exosomes were collected from control and Wnt4 over-expressing TECs. Transmission electron microscopy was used to visualize exosomes. Exosomal miR27b levels were measured by TaqMan qPCR, while Wnt4 protein content was assessed by ELISA. DiI-labeled exosomes were injected into mice intravenously to detect homing in the body. Using transmission electron microscopy exosomes were detected in the range of 50-100 nm as expected. TaqMan miRNA assay measured elevated miR27b levels, while ELISA showed high Wnt4-content in Wnt4-exosomes compared to control exosomes. To create a cellular aging model, steroid (Dx)-induced TECs were used. Dx accelerated aging, but Wnt4-containing exosomes could efficiently counteract Dx-induced senescence. Finally, in vivo injected DiI-labeled Wnt4-exosomes showed detectable homing to the thymus. According to our results Wnt4 and miR27b are both present in TEC exosomes. Our findings indicate that Wnt4 may act as an inhibitor of thymic involution via miR27b. However, further studies are required.

Hematopoiesis requires a self-generating and well-organized process of differentiation of hematopoietic stem cells to maintain cell life span and their diversity

Antonio Barać, Veronika Banović, Ana Jurić, Marija Lovrić

Medicinski fakultet Osijek

Disorders of normal differentiation of hematopoietic stem cells lead to three major types of hematological malignancies: leukemia, lymphoma and myeloma. Malignant hematopoietic diseases are associated with numerous molecular disorders involving: abnormal gene displacements caused by chromosomal translocations, inversions and duplications with the consequence of oncogenes activation, mutation and deletion of tumor suppressor genes. The development of molecular diagnostics has enabled the routine use of molecular methods in detecting malignant hematological disorders. Routine laboratory diagnostics include diagnosis and characterization of leukemia and lymphoma, monitoring of minimal residual diseases, prognosis of disease outcome and choice of therapy. The main and basic material for molecular diagnostics are nucleic acids (DNA and RNA) that are most commonly distinguished from bone marrow and peripheral blood samples. DNA is a stable analyte and desirable type of material for most polymerase chain reaction assays, DNA sequencing, mutation detection and genomic microarrays. The future of molecular diagnostics of hematological malignancies brings us new technologies that enable better sensitivity of nucleic acid measurements (digital PCR) and better mutation detection susceptibility (NGS-Next generation sequencing). Molecular diagnosis helps us understand molecular mechanisms and provides the ability to develop individual access to the patient giving effective therapy.

Effect of *Vipera ammodytes ammodytes* Snake Venom on the Human Cytokine Network

Ferenc Boda, Krisztina Banfai, Kitti Garai, Krisztian Kvell

Faculty of Pharmacy, University of Medicine and Pharmacy of Tirgu Mures

Introduction: Inflammation represents a defence mechanism of the body against irritating substances and harmful pathogens and is mediated through expression of pro- and anti-inflammatory cytokines. The type of expressed inflammatory mediators depends on the effect of the triggering substances on inflammation-related genes. Envenomation by snakes of the family Viperidae is known to induce local inflammation. This effect is attributed mainly to their content in metalloproteinases, phospholipase A2s and L-amino acid oxidases. The inflammatory effect caused by snake venoms has been associated with a significant increase of cytokines IL-1 β , IL-6, IL-8, IL-10 and TNF- α .

Materials and methods: To assess the impact of *Vipera ammodytes ammodytes* snake venom on the expression of inflammation-related genes we incubated U937 monocyte cells with different concentrations of snake venom solutions. Gene expression has been quantified using a TaqMan® Array Human Cytokine Network 96-well Plate in a RT-qPCR system, allowing the simultaneous determination of 28 different genes.

Results: At 1.0 $\mu\text{g/mL}$ concentration, the snake venom solution induced a marked change in the expression of several cytokine-related genes. Among the up-regulated genes, there were genes encoding interleukins, interferons and tumor necrosis factors, while three interleukin-related genes were found to be down-regulated.

Conclusions: Our results come as supportive information for the known effect of snake venoms on the human cytokine network. Furthermore, it provides new information regarding expression of genes not previously associated with the effect of snake venoms. Understanding the expression of mediators involved in the inflammatory response following envenomation could be of potential use in the development of targeted venom antiserums.

Muscle activation and movement pattern in bodyweight exercises

David Bodo, Marta Wilhelm

Physical Education and Sport Science, Faculty of Sciences, University of Pécs

Our study examines the process of maximal strength development using bodyweight exercises. Strength training with own bodyweight doesn't include external load, only uses the weight of the body in different positions, where the leverage between the supporting/hanging point and the center of mass of the body are changed. The most common strength training protocols use the percentage of maximal strength, with the one repetition maximum tested first. It is a simple method, when the trainer can calculate the quantity of the load (e.g.: 70% of 1RM). That method can be easily used on weight training, but with bodyweight exercises, it is more complicated, science exercises on different difficulty levels requires different positions and movement patterns. In our study, we aimed to measure the difference of forces created in muscles in push up variations, from easy to difficult exercises. To measure the peak load of the muscles, we used noraxon EMG on 12 muscles, on upper/lower extremity and torso. There were 5 male participants, age between 22-28 (24,9) years. Our hypothesis was that differences between the measured muscle activations of different push up variations mirrors the difficulty levels of the exercises. Although, there was no significant difference (only tendency), our results suggests that there is a muscle activation pattern, that highlights the quality of the movement. In this case, technically perfectly preformed exercises has similar muscle activation pattern in very different levels of push up variations. Further goal is to find the relation between movement patterns and muscle activation in calisthenics exercises, and create an appropriate process to value the difference of forces in muscles.

Analysis of temozolomide resistance in glioblastoma cell lines

Barbara Brandt, Ibolya Koloszar, Hajnalka Les, Marica Nemeth, Marianna Pap

*Department of Medical Biology and Central Electron Microscope Laboratory, Medical
School, University of Pécs*

Background: Glioblastoma multiforme (GBM) is a type of glioma, which represents the most common and aggressive primary brain tumor in the central nervous system. This tumor is usually highly invasive, therefore it has a very poor prognosis with an average survival of about 14 months. Temozolomide (TMZ) is a brain-penetrant alkylating agent used to treat GBM and astrocytomas. The resistance to TMZ is a major problem in the treatment of GBM and it is due to the over-expression of O6-methylguanine-DNA methyltransferase (MGMT). The aim of our study is to find a clinically effective combination treatment for glioblastoma, and to characterise our glioblastoma cell lines, determine their rate of proliferation and MGMT expression.

Materials and methods: Different GBM cell lines were cultured. The rate of proliferation were determined by counting the cells and the cell viability was tested by ATP assay. The MGMT protein expression was determined by immunocytochemistry and Western blot analysis. GBM cell lines were treated with different temozolomide and tunicamycin concentrations. The treated cells viability were determined by ATP assay.

Results: Most of the GBM cells have responded to higher concentrations of temozolomide and tunicamycin. MGMT expression were detected in every cell line, although the different GBM cell lines don't express the MGMT protein in the same quantity.

Zika virus replication in human blood platelets

Dora Buzas

Virological Research Group, Faculty of Sciences, University of Pécs

The role of platelets in hemostasis is well described, but its function in immune reactions and host defense have received less attention. Viral infections often cause thrombocytopenia, a multifactorial state when platelet count in blood is largely decreased. In this study we followed the replication of ZIKV in human platelet isolates.

Platelets are anucleated cell fragments derived from megakaryocytes of the bone marrow. Broad repertoire of surface receptors enable them to attach each other and form a blood clot upon tissue damage. Other surface receptors have a role in the modulation of inflammatory reactions and immune responses. It has been shown for different viruses (for example Dengue fever virus) that they can attach to platelets through surface receptors. However virus – platelet interactions are not well described and numerous virus families were not examined for the ability of hijacking platelets.

Zika virus (ZIKV) is an enveloped, positive-sense single-stranded RNA virus, that belongs to the Flaviviridae family. ZIKV is naturally transmitted by infected female mosquitoes of the *Aedes* genus, although several human to human transmission routes were also described such as sexual transmission.

In this study we followed the replication of ZIKV (strain: Zika virus 976 Uganda) in human platelet isolates by negative strand-specific quantitative reverse transcription-polymerase chain reaction and we detected viral RNA in platelet culture 72 h post-infection, that is a direct proof of viral replication. From these results we postulate that ZIKV could replicate its viral RNA by utilizing the translational machinery of platelets.

Toward understanding the binding of a polyene antifungal drug to serum albumins

Diana Derdak, Miklos Poor, Sandor Kunsagi-Mate, Beata Lemli

Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Pécs

Due to the fact that human organizations are resistant to the main group of antifungal drugs, the treatment of fungal infections represents a major challenge nowadays. Moreover, the rapid increasing of the presence of antibiotics in the environment is also an important public health concern. Amphotericin B is a polyene antifungal agent which is used in the treatment of systemic fungal infections. It could be get absorbed in the blood circulation system in humans, and form complex with the main transporter proteins, like serum albumins. Amphotericin B forms aggregates and this molecular organization can be easily detected by spectroscopic methods. Further this phenomenon, the drug plays an important role of its effectiveness and toxic side effect. As it is described in the literature, Amphotericin B is most likely bind to albumin in monomeric form. Only little information about the physical chemical background of the complex formation is available yet. Therefore, in this work we investigated the interaction between amphotericin B and human-, bovine- and fatty acid free bovine serum albumin, using optical spectroscopic (UV-Vis, Photoluminescence, Fluorescence polarization) and differential scanning calorimetric methods. Our aim was to get deeper insight to the molecular mechanism of the interaction between amphotericin B and serum albumins including the binding and transition properties. The study of these protein-drug interactions may provide knowledge of the binding, thermodynamics and kinetics features, which are related to the therapeutic efficiency of the drug.

This work was supported by the GINOP-2.3.2-15-2016-00049 grant.

Removal of zearalenone and its metabolites from aqueous solutions by insoluble β -cyclodextrin bead polymer

Zelma Faisal, Afshin Zand, Timea Bencsik, Lajos Szente, Miklos Poor

Department of Pharmacology, Faculty of Pharmacy, University of Pécs

Zearalenone is a *Fusarium*-derived mycotoxin, which expresses strong xenoestrogenic effects in animals and humans. During the biotransformation of zearalenone, its reduced metabolites α -zearalenol and β -zearalenol are formed. These metabolites are also active, α -zearalenol is even more toxic than the parent compound. Zearalenone mainly appears in cereals and cereal-based products, including beer. Cyclodextrins are ring-shaped host molecules, built up from glucose units. The external part of cyclodextrins is hydrophilic, while the apolar cavity can accommodate hydrophobic guest molecules. Since cyclodextrins are able to form relatively stable complexes with some mycotoxins, it is reasonable to hypothesize the potential suitability of cyclodextrins as mycotoxin binders.

In this study, the removal of zearalenone, α -zearalenol, and β -zearalenol from aqueous solutions by insoluble β -cyclodextrin bead polymer (BBP) was investigated. Time-dependence, dose-dependence, pH-dependence, and temperature-dependence of mycotoxin removal was tested, after which the residual mycotoxin content was analyzed by HPLC-FLD. Then, the regenerability and reusability of BBP were examined as well. Finally, the removal of zearalenone from corn beer was investigated.

Rapid and concentration-dependent removal of zearalenone and zearalenols from aqueous solutions by BBP was observed. Temperature-dependence of the removal was not significant, while BBP was more effective at acidic and neutral pH compared to alkaline circumstances. Regeneration of BBP by ethanol-water mixture was successfully performed, after which BBP was applied with the same efficiency than first time. Furthermore, BBP effectively removed zearalenone from spiked corn beer samples. In conclusion, BBP seems a promising tool to remove zearalenone (and/or its metabolites) from aqueous solutions, including different drinks.

The Effect of Ozone on Forensic Samples

Vivien Fejes, Viktor S. Poor

Department of Forensic Medicine, Medical School, University of Pécs

The most abundant sample type in forensic genetic analysis is the blood. The DNA is sensitive to environmental factors (such as oxidative stress or radiation). Ozone is a strong oxidant, it can oxidize lipids and proteins in the cells, furthermore, it may produce free radicals. Because of the low price and easy implementation, it is used as a bactericide agent since decades, nowadays especially for car interior and air conditioner system disinfection. The aim of this study is to investigate whether the commonly used quick, only 30 minutes long car cleaning procedure with ozone has an impact on the blood samples, in particular on the DNA content. Before the ozone treatment, we put 3 blood samples to different places in the car. After the treatment, we performed preliminary investigations to detect blood with luminol and hemoglobin test. The DNA was isolated with Chelex, and we compared the DNA quantity and quality of the treated and untreated samples with qualitative real-time PCR. During the examination with luminol, we observed a slight difference in the color reactions. The hemoglobin tests gave uniformly positive results. In the quantitative PCR, we tested a shorter and a longer product. Product length does not affect significantly the DNA quantity, however we found difference in the quality, the degree of degradation showed variability between the treated and untreated samples. According to our result, we conclude that the ozone treatment has influence on the condition of the DNA.

Optimization of determination of total polyphenol content in *Origanum vulgare* L.

Krisztina Ferenczi, Timea Bencsik

Faculty of Pharmacy, University of Pécs

Introduction: *Origanum vulgare* L. (oregano) is a popular spice nowadays, its essential oil components are responsible for its antiseptic and expectorant effects, its polyphenols are antioxidant. In the literature, there are many publications referring to the examination of the polyphenol content of oregano; however, the authors usually use different solvents, dilutions, and concentrations of the reagents, so the available data are not comparable with each other. The aim of our work was to determinate the total polyphenol content of this plant among different conditions and compare the results.

Materials and methods: For the experiments, the spectrophotometric method official in the VIII. Hungarian Pharmacopoeia was used with different modifications. During the extraction, we applied various solvents (distilled water, 10%, 25%, 50%, 75%, and 96% ethanol), different methods (24 h at room temperature, 10 min and 30 min in ultrasonic water bath at room temperature, and 30 min in water bath at 70°C). The total polyphenol content was determined with a UV-VIS spectrophotometric method at 760 nm. For the determination, Folin-Ciocalteu reagent and Na₂CO₃ solution were used.

Results: In all methods, the 50% ethanol extracts showed the highest total polyphenol content and the 96% ethanol extracts showed the lowest values. The best methods were the 30 min long extraction with ultrasonic water bath at room temperature and 30 min long extraction with water bath at 70°C. The efficiency of these two methods was very similar. In the literature, the length and temperature of the incubation is varying between 30-120 min and 25-50°C, but our experiences show that it is not worth incubating the samples for more than 30 min and at higher than room temperature. In the publications, generally four different wavelengths (725, 750, 760, 765 nm) are used for the measurement of the absorbance; however, according to our results, the absorption maximum is 732 nm.

Patient experience and attitude toward medicines and dietary supplements purchased outside the traditional supply chain

Andras Fittler, Robert Gyorgy Vida, Matyas Kaplar, Eszter Jahn

*Department of Pharmaceutics and Central Clinical Pharmacy, Faculty of Pharmacy,
University of Pécs*

BACKGROUND: The growing market of online pharmacies has caused numerous patient- and medication safety concerns for health systems.

PURPOSE: A complex methodology called Risk Based Safety Mapping of Online Pharmaceutical Market has been developed to evaluate patient safety threats. The aim of our current study is to evaluate actual patient safety risk in outpatient setting and integrate this data into this methodology.

MATERIALS AND METHODS: A Hungarian survey questionnaire was developed for this study after the completion of a pilot study (n=100). Data were collected directly from Hungarian citizens from Southern Transdanubian region who use outpatient health service for chronic or acute conditions. Five sections covered the 28 item questionnaire: (1) Medicine purchase; (2) Online medicine purchase experiences, attitude; (3) Internet use; (4) Health state and medications; (5) Demographics. Study protocol was approved by the Regional Committee for the Research Ethics (approval number: 6835).

RESULTS: The sample of the present study consisted of 1055 participants, 456 (43,2%) responders reported to have chronic health conditions. The correspondence of factors of internet usage and online drug purchase was examined. The attitude reactions about the online medicine purchase was weighted and a relative attitude rate was computed. (Mean= -0,37 SD=1,2) There is a weak still significant tendency toward rejection. According to our model the time spent on the internet predicts the willingness of online purchase in general ($R^2=0,41$) and tendency of online self-diagnosis ($R^2=0,29$). Online self-diagnosis predicts willingness of independent medication ($R^2=0,24$). The three factors of relative attitude, general online purchase and independent medication explains 36% of the variance of tendency to purchase drugs online.

CONCLUSIONS: There is an increasing risk of patients consuming dietary supplements or potentially counterfeit medications purchased over the Internet.

Analysis of capsaicin and dihydrocapsaicin metabolism of the small intestine in the diabetic rat by HPLC-FLD

Eszter Fliszar-Nyul, Attila Almasi, Edina Pandur, Ramona Pap, Kitti Garai, Krisztian Kvell,
Monika Kuzma, Gyula Mozsik

Institute of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Pécs

A sensitive and selective HPLC-FLD method has been developed for determination of capsaicin (trans-8-methyl-N-vanillyl-6-nonenamid) and dihydrocapsaicin (8-methyl-N-vanillylnonan-amide) in samples generated in rat small intestine luminal perfusion experiments. Extrahepatic metabolism – including intestinal metabolism - may also significantly influence the disposition of the xenobiotics. These experiments were designed to study the biotransformation of capsaicinoids and the luminal appearance of their metabolites in rat proximal jejunum which was perfused with isotonic medium containing 100 μ M Capsaicin natural (purchased from Sigma Aldrich). Diabetes was induced in the experimental animals by pretreatment with 65 mg/kg streptozotocin 7 days before the intestinal perfusion. The chromatographic analysis of the perfusates was performed at room temperature on a Kinetex® C8 column using isocratic elution with a mobile phase consisting of formic acid solution (pH 2.6) and acetonitrile (60:40, v/v) with a flow rate of 0.7 ml/min. Fluorescence detection was performed at excitation and emission wavelengths of 230 and 323 nm, respectively. The method was evaluated for a number of validation characteristics (selectivity, repeatability and intermediate precision, LOD, LOQ and calibration range). The method was successfully applied to the investigation of the intestinal metabolism of capsaicin and dihydrocapsaicin and the luminal appearance of capsaicinoid metabolites in rat jejunum perfusates. Identification of the structure of the metabolites was based on MS/MS analysis.

Immunosenescence and inflammaging: an intricate connection

Tamas Fulop

*Research Center on Aging, Faculty of Medicine, Université de Sherbrooke,
Sherbrooke, Qc, Canada*

One of the most notable biological age-related changes are alterations in the immune system. Near constant antigenic stimulation by various antigens such as chronic viral infections, altered proteins, and/or tumoral cells is characteristic of this change. The innate part of the immune response is chronically stimulated at baseline and is in a state of alertness to respond to various stimulation. This is most of the time assured by the trained memory of the innate cells. Although the effectiveness of innate immune functions are decreased, this state of activation assures a higher adaptive response. This chronic low grade inflammation is stimulating/exhausting the adaptive immune system which itself is constantly under the attack of the chronic antigenic stress. The induction of these adaptive responses will be characterized and the intracellular pathways leading to immunosenescence, which further stimulates innate responses, will also be discussed.

Exercise alleviates senescence-related parameters in healthy, young individuals

Kitti Garai, Krisztina Banfai, Adam Gyebrovski, Zoltan Adam, Emese Katai, Tamas Nagy,

Judit E. Pongracz, Marta Wilhelm, Krisztian Kvell

Institute of Pharmaceutical Biotechnology, Faculty of Pharmacy, University of Pecs, Hungary

Studies suggest that regular physical activity effectively restores immune functions, particularly in the elderly. However, no study has been conducted to investigate the effect of lifestyle intervention on young and healthy, previously inactive individuals. We have aimed for this and employed a multidisciplinary approach. We have examined the effect of a six-month long lifestyle intervention on physiological (blood pressure, heart rate, 6-minutes walking test, relative VO₂max) and immunological parameters (T-cell receptor excision circle or hTREC copy number, cytokine profile) in young and healthy, previously inactive individuals. All parameters have been assessed before the intervention, after three months and six months. Actigraph devices recorded the intensity and duration of physical activity. Levels of blood parameters including cortisol and CRP from serum were also measured. TNF- α concentration from serum exosomes was determined using CBA and flow-cytometry. Exosomal miRNA was evaluated using NanoString miRNA panel. hTREC copy numbers were measured by 3D-PCR. Our results show that the six-month regular exercise program significantly improved the physiological parameters such as resting systolic blood pressure and performance (6MWD). Certain hormonal and other blood parameters have also ameliorated. Regular exercise lowered the concentrations of TNF- α and CRP. Exosome miRNA profiling also showed a significant number of microRNAs being altered and moderate exercise increased hTREC levels as well after 6 months. In summary, using numerous techniques we identified molecular changes triggered by moderate and regular physical activity that can directly delay senescence.

Ferokinetics – Diagnostic value of laboratory tests

Ivana Gašpar, Antonio Barać

Mediinski fakultet Osijek, Osijek

Iron is an essential oligoelement that participates in numerous biological processes. The regulation takes place on several levels and precisely regulates the relationship of input and iron requirements. Coding genes that control kinetics of iron in the body must be aligned with other regulatory systems so as to avoid iron associated disorders. Any deficiency or excess of iron in the body can lead to a homeostatic imbalance manifested in different ways and can lead to life-threatening conditions. For this very reason it is important to improve the iron metabolism monitoring system. The laboratory test used for monitoring are: measurement of serum iron concentration, concentration of UIBC (unsaturated iron binding capacity) and TIBC (total iron binding capacity), ferritin, transferrin and soluble transferrin receptors. Cytochemical staining of bone marrow punctures with purine blue are also used. Nuclear test which investigates the distribution and fate of iron in the body after the injecting of radioactive iron (^{59}Fe) is called ferokinetics. Every test targets different point in the circulation of iron. It is important to know the benefits and limitations of each test to correctly interpret the results for better differential diagnosis that contributes to overall patient health improvement.

Key words: iron, ferokinetics, laboratory tests

Investigating the everyday life of people with Post-Polio Syndrome

Szabina Hodovan

Faculty of Health, University of Pécs

Introduction: Of the survivors of polio, 15 to 40 years after infection, most of them have one or more of the symptoms or symptom of the post-polio syndrome. The aim of our study is to get acquainted with the hospital's supply, treatment and rehabilitation skills in the early stages of the disease through our questionnaire. Another objective of our research is to evaluate the factors affecting everyday activity among polio survivors.

Material and Method: A retrospective and prospective, cross-sectional, quantitative study was conducted between February 2017 and March 2018 among polio survivors in Hungary (n = 98). The sample selection was done with a targeted, expert selection method. For our questionnaire we have the Polio Survivors Network - My Polio Life questionnaire and Polio NZ. Inc. - Polio NZ. Inc: questionnaire-Who validated the disease specification. We used the individual questions of post-polio syndrome and the individual questions generated by the hypotheses. Self-evaluation was measured using the Rosenberg self-assessment questionnaire. As a statistical method we used X 2 probes and personal correlation analysis using MS Excel 2016 and SPSS 22 programs.

Results: The disease limiting factor in the continuation of the chosen hobby was a significant difference ($p = 0.049$) for the sexes. Furthermore, sporting activities were limited by age, and gender differences were significant ($p = 0.030$) in sporting activities. Out of the participants 64% (n=63) of respondents were married, cohabiting or socially involved, and 61% (n=60) of those have secondary vocational, high school or higher education qualifications.

Conclusions: Post-polio syndrome sufferers have a decrease in physical activity as age progresses. A major problem is the feeling of weakness in muscles that have been damaged in the past, as well as the sensitivity to cold and, as age progresses, the need for mobility aids has increased. Better rehabilitation treatments and wider access to mobility tools would greatly improve the quality of life for the survivors.

Keywords: polio virus, post polio syndrome, quality of life.

The Ministry of Human Resources ÚNKP-17- 1-I. code of the New National Excellence Program.

Could irradiation be more efficient on breast cancer cell-line?

Orsolya Homoki

Institute of Anatomy, Medical School, University of Pécs

Breast cancer is one of the most common cancerous disease which affects every 8th women according to statistics. Agents effecting the radio-sensitivity of the cancer are used more and more in oncological treatments. Irradiation is a common treatment in breast cancer, that is why we are examining the irradiation sensitizing effect of the Pituitary adenylate cyclase-activating polypeptide which is a PAC1 receptor antagonist. We used MCF7 (ATCC) cell-line between experimental conditions which is an adenocarcinoma according to histology. 2Gy dose was applied with telecobalt machine following a 72 hour pretreatment of 2uM PACAP6-38. The survival of the cells was detected with MTT test, Muse Annexin Dead Cell Assay Kit was used to measure the apoptotical changes. R&D Homan Apoptosis Array Kit and Human Phospho-MAPK Array Kit were used to analyse the occurred molecular biological changes. PACAP6-38 has a cell-survival reducing effect in 2 uM concentration. The pretreatment significantly ($p < 0,05$) increased the ratio of cells suffering early or late apoptosis comparing to the control group receiving only irradiation. As a result of pretreatment, increase of activation of proapoptotic factors (Akt1, Akt2, Akt3, CREB, JNK1, JNK2, JNK3) and decrease of antiapoptotic factors (HIF1-alfa, Survivin, Livin) was detected in the groups receiving pretreatment and groups who received pretreatment followed by irradiation comparing to control groups. Our investigation may lead to the PAC1 receptor antagonist PACAP6-38 pretreatment is able to make MCF7 cells more susceptible to irradiation even in a smaller dose than therapeutic dose. In the following we are planning to analyse the occurring molecular biological changes.

Effects of different parameters of herbal tea preparation on active compounds of *Silybi mariani fructus*, *Uvae ursi folium*, and *Rosae pseudofructus*

Adrienn Horvath, Timea Bencsik, Gyorgyi Horvath

Department of Pharmacognosy, Faculty of Pharmacy, University of Pécs

Introduction: One of the oldest and most popular methods for preparation of herbal extracts is the preparation of a tea. Our aim was to examine, how different parameters of the preparation influence the amount of active compounds in teas of three commercially available drugs official in the Ph. Eur., namely *Silybi mariani fructus*, *Uvae ursi folium*, and *Rosae pseudofructus*.

Materials and methods: For preparation of water extracts, we used three different conditions: hot water for 5 min, room temperature for 2 hours, and room temperature for 10 hours. HPLC was used for determination of silybinin, arbutin, and hydroquinone contents; and spectrophotometric methods were used for determination of total tannin and vitamin C contents.

Results and conclusions: Nearly no silibinin could be detected in the teas of *Silybi mariani fructus*; so it is surprising, why this drug is commercially available for tea preparation, if its hepatoprotective compounds are not water soluble. The best method for extraction of vitamin C from *Rosae pseudofructus* is the soaking for several hours at room temperature. Because of their accelerated lifestyle, most of the people use hot water for 5-10 min, thereby reducing the effectiveness of the therapy. Additionally, we found that some distributors's products contain the rosehip without comminution, and in these samples, no vitamin C could be detected. The arbutin and hydroquinone content of *Uvae ursi folium* increased with the longer time of extraction, but the tannin content (responsible for side effects) also increased parallelly, so changing the time and temperature of extraction is not appropriate for avoidance of side effects.

References: 1. Council of Europe. European Pharmacopoeia. 6th ed. Strasbourg: Council of Europe; 2007.

Extracellular vesicles and Wnt signaling in the background of Chronic Obstructive Pulmonary Disease

Lina Hudhud, Elhusseiny Mohamed Abdelwahab, Krisztina Banfai, Kitty Garai, Judit E. Pongracz

Department of Pharmaceutical Biotechnology, Faculty of Pharmacy, University of Pécs

Background: Chronic Obstructive Pulmonary Disease affects the large and small airways. According to the WHO, COPD is the third cause of mortality globally. Wnts are lipoglycoproteins that play important role in different cellular functions such as proliferation and differentiation. Wnt ligands are associated with human diseases including inflammation that can be triggered by Wnt5a. By binding to membrane receptors, Wnt proteins start an intracellular signaling cascade. As extracellular vesicles, exosomes are known to transport signals amongst cells and tissues. Oncosomes are large extracellular vesicles that believed to be released from cancer cells. As Wnt molecules are glyco-lipoproteins, they are transported in EVs locked into the lipid bilayer of the vesicles. Aim: This study aimed to determine if Wnt5a is present in extracellular vesicles in sera of COPD patients. Method and Results: EVs of COPD patients and healthy controls were isolated as well as cell line supernatants. The isolated EVs were visualized by electron microscopy (EM). Lung cancer A549 and the Wnt5a overexpressing A549 cell lines were used as positive controls. Wnt5a protein in EVs was studied by western blotting and ELISA. EM revealed that exosomes were present in diseased cell lines supernatant. The expression of Wnt5a was upregulated in COPD patient's sera compared to healthy individuals. Conclusion: The inflammation inducing Wnt5a protein is present in EVs of COPD patients. As EVs are transported all over the body, our finding might explain the systemic inflammatory nature of COPD.

Problems with the safety testing of biopharmaceuticals

Mary Keen

Keynote Speaker of Pharmaceutical Sciences Session

Biopharmaceuticals make up an increasing proportion of new drugs on the market and under development. These drugs differ from traditional, small molecule drugs in that they are recombinant proteins or nucleic acids. One advantage of this type of drug is that they can be extraordinarily selective, often only producing effects in their target species. However, this raises problems for safety testing: how can you ensure that a drug is safe to use in humans if it only produces effects in humans?

Study on interaction of reduced glutathione (GSH) with some Chalcone analogues

Fatemeh Kenari, Pal Perjési

Institute of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Pecs

Introduction: Chalcones are intermediate compounds of the biosynthetic pathway of the natural flavonoids. The wide range of biological activities of both naturally occurring and synthetic analogues, among others the cytotoxic, antitumor, anti-inflammatory, and chemopreventive (antioxidant) properties are well documented in the literature. Chalcones are α , β -unsaturated ketones, and representatives of this class of compounds have demonstrated a preferential reactivity towards thiols. In our earlier experiments some cyclic chalcone analogues showed a significant effect of GSH status of Jurkat T lymphocyte cells.

Methods: In this experiment in vitro interactions of 4'-substituted chalcones and their cyclic analogues (ring size = 5, 6 and 7) was studied under controlled (pH, T) conditions. The incubations have been performed at three different pH (pH 3.2, 6.3 and 8.0) at 310 K temperature. For monitoring the process of the reactions a RP-HPLC-UV-VIS method has been developed. By means of the analytical method, partial separation of the formed diastereomeric adducts was also possible.

Results: The results indicate that each investigated chalcone participate in an addition reaction with GSH. The reactions are reversible and lead to form equilibrium mixtures. The rate of reaction shows strong pH dependence, ionization of GSH increases the reaction rate. The reaction leads to form diastereomeric chalcone-GSH adducts. The ratio of the integrated area of the chromatographic peaks corresponding to the cis and trans diastereomeric pairs changes as a function of incubation time. The kinetic products are converted into the more stable thermodynamic ones through the reversible reaction.

References: Zs. Rozmer, P. Perjési P: Naturally occurring chalcones and their biological activities *Photochem. Rev.* (2016) 15, 87-120. A. Bernardes; C. N. Pérez; M. Mayer; C. C. da Silva; F. T. Martins; P. Perjési: Study of reactions of two Mannich bases derived of 4'-hydroxychalcones with glutathione by RP-TLC, RP-HPLC and RP-HPLC-ESI-MS analysis. *Braz. Chem. Soc.* ([dx.doi.org/10.21577/0103-5053.20160260](https://doi.org/10.21577/0103-5053.20160260)).

Examination of BFSP1 (Beaded Filament Structural Protein 1 or Filensin) tumor specific splice variants in ex vivo human tumorous tissue and human serum samples

Bence Kiss

Institute of Biochemistry and Medical Chemistry, Medical School, University of Pécs

BFSP1 (Beaded Filament structural Protein 1, or Filensin) is an eye lens specific cytoskeletal protein forms intermediate filaments (IFs) with its assembly partner (BFSP2). Preliminary experiments have proved the evidence that BFSP1 is expressed in various cancer cell lines as well. Here, we show the presence of BFSP1 in ex vivo human tumor samples, compared to non-tumor tissue samples by Western Blotting, MS and immunohistochemistry. Furthermore, we quantitatively examined BFSP1 concentration in human sera pre-op, as well as post-op after 1 and 3 weeks. We performed qPCR in order to detect different splice-variants of the protein from human ex vivo tumor and serum samples, respectively. Furthermore, recombinant BFSP1 splice-variants were immobilized and incubated with cancer cell lysates in order to determine the possible interacting proteins with BFSP1 (MS). Since there are few antibodies against BFSP1 and there is no antibodies recognizing only the splice-variants of BFSP1 respectively, we immunized rabbits with a BFSP1 antigen presenting self-assembling nanoparticle (SANP). This new antibody will be essential for further research like detection of the different splice-variants in human tumorous tissue and serum. According to the literature, BFSP1 has been known as a cytoskeletal protein expressing particularly in eye lens so far. The presence of BFSP1 in cancer cells seems unlikely and it indicates a new exciting approach in the field of tumor biology. To establish the possible role of a new cytoskeletal protein as a tumor marker might have extraordinary significances in cancer diagnosis.

Steroid-harboring nanoparticles provide anti-inflammatory response with less adverse effect

Barna Kollar, Krisztian Kvell

Institute of Pharmaceutical Biotechnology, Faculty of Pharmacy, University of Pecs, Hungary

Steroids are acknowledged anti-inflammatory drugs used in multiple conditions, including autoimmune disease. Steroids provide strong suppression of inflammation, however, their long term utilization triggers numerous adverse effects including obesity, diabetes, osteoporosis, edema retention etc. As a result, only inflammatory flares are treated with steroid compounds, for short term. Our collaborative research team has produced nanoparticles of specific size harboring steroid compounds. In theory, due their specific size steroid-harboring nanoparticles trigger phagocytosis in monocytes and macrophages, but leave other (non-phagocytic) cells unaltered. Our human in vitro data indicate that steroid particles show potent anti-inflammatory effect on monocytes / macrophages, equivalent to that of steroid solution. However, their adverse effects are reduced using non-phagocytic cells. Liver cells, for example, show increased viability with steroid particles as opposed to steroid solution. Our working hypothesis was that steroidparticles of a specific size range can preferentially target monocytes / macrophages, the major mediators of inflammation. Other (nonphagocytic) cell types shall largely be unaltered by steroid particles, as opposed to steroid solution. This is confirmed by our data. Our technology allows for the production of regular steroid compounds with significantly reduced side effects, with the promise of long-term use in human.

The performance improving effect of swimming in synchronized swimming

Vivien Laski, Rita Soos, Adam Gyebrovski, Alexandra Cselko, Marta Wilhelm

Institute of Sport Sciences and Physical Education, Faculty of Sciences, University of Pécs

In synchronized swimming high level of technical and conditional abilities are needed, and well planned swim trainings are behind it. Based on these the aim of our study was to show that (i) well planned swim trainings are decreasing the effort needed for the proper technical performance of basic elements like vertical or barracuda and (ii) to find the components of body structure, body composition influencing the elucidation of these technical elements.

In our study elit synchronized swimmers participated ($N=47$, 15.35 ± 1.19 years), training 4 times a week, training 3hrs. The measurements were carried out twice, first in august 2017, and 8 months later. Body composition was measured, muscle force with dynamometer with each hand. After a standard warm up 4x100 freestyle and the 200m medley swimming was measured. Before and after the swim test, blood lactate levels were measured from fingertips at 1, 3 and 5 minutes after the loads. Barracuda and vertical elements were performed, and then five well-trained competition judges independently clarified them.

Their body composition did not change significantly during the studied period. Lactate values before and after loading changed significantly, the highest value was at 1 minute following the test. Time of 200m medley swimming improved significantly ($p = 0.02$). Correlation was found between the vertical height and the hand grip ($r=0,76$).

The average score of the measured technical elements: body boost 6.82, barracuda 6.83, vertical position 6.18. There is a negative correlation between the 4x100m freestyle and vertical score ($r = -0.63$), while the 200m mixed and vertical is $r = -0.71$, meaning that force strongly influences vertical execution. A negative correlation between the barracuda and-4x100m freestyle swimming ($r = -0.50$) and 200m medley swim ($r = -0.54$) was also found. Barracuda correlates with vertical position ($r = 0.64$).

Epilepsy-Surgery?

Katalin Nora Lorincz, Janszky József, Gyimesi Csilla

Doctoral School of Clinical Neurosciences, Medical School, University of Pécs

Introduction: Epilepsy as a chronic, potentially severe neurologic disease significantly influences the quality of life of the epileptic patients. In candidates well selected for surgery, the seizure freedom is realistically achievable, and the quality of life can be further improved with complex individual rehabilitation.

Objective: We aimed to evaluate the postoperative outcome of patients underwent epilepsy surgery between 2005-2016 at the Epilepsy Center at Pécs, with special emphasis on their social and employment status after surgery.

Methods: We evaluated seizure status at regular follow up visits after surgery and the quality of life using questionnaires focusing on employment and social status.

Results: 76% of the 72 patients underwent resective epilepsy surgery were free from disabling seizures (Engel class. I), and 10% had rare disabling seizures (almost seizure free) (Engel class. II), 7% experienced worthwhile improvement (Engel class. III) and 7% had no worthwhile improvement (Engel class. IV). 67% of patients grouped Engel class.I, 34% of patients of Engel class. II, and 20% of patients of Engel class. III group were employed, while patients in Engel class. IV group were not employed.

Conclusion: Our results not only resemble the international tendencies and success rate, proving epilepsy surgery as an available, valid and effective treatment in well selected patients, but also demonstrate the need of organizing complex individual postoperative rehabilitation in Hungary raising further questions to discuss.

The effects of non-caloric artificial sweetener consumption on Microbiom

Judit Magyar, Eva Polyak

Institute of Nutritional Science and Dietetics, Faculty of Health Science, University of Pecs

Recently, much attention has been paid to the regulating effect of the gut microbiota on the host health. The microbiom plays an essential role in the host-gut interactions, like metabolism, immune response, and has a crucial role in food digestion, energy homeostasis in the human body and weight control.

It can be easily disrupted by many factor, such as diet and its components, antibiotics, heavy metals.

Distruptions in the microbiom or dysbiosis, have been linked to numerous metabolic disease states including obesity, diabetes, or inflammatory bowel disease.

Perturbation of gut barrier homeostasis is also responsible for changes in microbiom diversity and function.

The effects of artificial sweetener consumption are controversial, numerous studies have indicated its effective role in weight loss in individuals with type 2 diabetes mellitus and impaired glucose tolerance, other studies showed that it causes weight gain and metabolic derangements.

In our review I would like to demonstrate the result of the effects of artificial sweetener consumption.

Effects of quercetin, chrysin, and their metabolites on the xanthine oxidase-catalyzed biotransformation of 6-mercaptopurine

Violetta Mohos, Attila Panovics, Miklos Poor

Department of Pharmacology, Faculty of Pharmacy, University of Pécs

Xanthine oxidase (XO) is a non-microsomal enzyme responsible for purine catabolism. Allopurinol is an effective inhibitor of XO used for the treatment of hyperuricemia. XO plays an important role in biotransformation of the antitumor agent, 6-mercaptopurine (6-MP). Simultaneous administration of allopurinol and 6-MP leads to the slow elimination of 6-MP, resulting in unpleasant or even toxic consequences. Previous reports highlighted that some flavanoids (including quercetin and chrysin) are strong inhibitors of XO. Quercetin and chrysin appear in several plants and dietary supplements; however, due to their significant presystemic elimination, mainly their metabolites achieve high concentrations in blood and tissues. In the present study, the inhibitory effect of quercetin and chrysin metabolites on XO was tested, using xanthine (endogenous substrate of XO) and 6-MP as substrates.

Glucuronide conjugates (quercetin-3-glucuronide, isorhamnetin-3-glucuronide, and chrysin-7-glucuronide) were poor inhibitors of XO. However, significant inhibition of XO by quercetin, chrysin and their methylated (tamarixetin, isorhamnetin) and sulfated (quercetin-3'-sulfate, chrysin-7-sulfate) metabolites was observed. These compounds showed stronger effect on XO-catalyzed 6-MP oxidation than the positive control allopurinol. Furthermore, some flavonoid metabolites showed more potent inhibitory action, even compared to their parent compound. IC₅₀ values of flavonoids in xanthine and 6-MP assays were very similar, while allopurinol was much weaker inhibitor of 6-MP compared to xanthine oxidation.

Our results demonstrate that some flavonoid metabolites are able to strongly inhibit the biotransformation of 6-MP. Therefore, we need to consider the potentially hazardous consequences of the simultaneous administration of 6-MP and dietary supplements with high flavonoid content.

Current trends in Zika virus research

Henrietta Papp, Dora Buzas, Ferenc Jakab

Virological Research Group, Faculty of Sciences, University of Pécs

Zika virus (ZIKV) was one of the most emerging viruses in recent years. It was first isolated in 1947 in the Zika forest in Uganda from a rhesus monkey. Since then, Zika virus infection was detected in different parts of the globe and caused mild and severe diseases such as microcephaly and Guillain- Barré syndrome in humans. In 2016 World Health Organization declared the Zika virus infection as a public health emergency. Thereupon the global scientific community started to investigate more intensively its pathomechanism, epidemiology, molecular biology and to develop therapies against it. Human cases and experiments on mice and non- human primates showed that the Zika virus exhibits broad tissue tropism, it can cross the blood- brain barrier and infect neural progenitor cells as well as mature neurons. It also targets the circulating monocytes thus spreads easily to the different parts of the body. As a result, it can also invade the reproductive organs and infects for instance spermatogonia, Sertoli cells in males or the vaginal epithelium, Hofbauer cells, trophoblasts in females and the cells of the developing fetus. Despite the extensive studies, direct therapeutics and vaccines for Zika virus infection are not yet available. On the other hand, it was observed that ZIKV preferentially targets brain tumor cells relative to normal neural cells. Overall more studies are needed to treat the infection or even to utilize the virus against malignant tumors.

Examination of biologically relevant electron transfer in photolyases with ultrafast laser spectroscopy

Jonatan Pasitka

Department of Biophysics, Medical School, University of Pécs

In the last few decades an enormous technological expansion could be seen in the field of lasers. This resulted in the application of basically biophysical methods in the every day medicine, methods like super-resolution microscopy, CARS microscopy, Raman-spectroscopy and even ultrafast laserspectroscopy. With the help of ultrafast laser spectroscopy the recording of intramolecular procedures became possible even on the scale of femtoseconds. Several, biologically relevant processes are known that take place within this scale. Such example is the photoisomerisation that means the basics of vision or the redox potential changes in photolyases and cryptochromes leading to their proper functioning. We have been focusing on the ultrafast laser spectroscopic features of these latter two proteins. They are phylogenetically connected, however photolyase is a DNA repair enzyme while cryptochrome is the member of the group of proteins composing the circadian clock. The explanation for such different functions so far from each other lays in the understanding of the procedures happening intramolecularly on the scale of picoseconds.

Evaluation of modified-release formulation containing *Silybum marianum* herb extract for oral application

David Sinka

Department of Pharmaceutical Technology, University of Debrecen

Silymarin is the extract of active ingredients of the plant *Silybum marianum*. It is used in medicine for curing or preventing liver and gall diseases for thousands of years. Silymarin has a low solubility and permeability, belongs to BCS IV. Its current pharmacotherapy means conventional dosage forms such as capsules, but it results in low bioavailability. Our main goals through the research were to formulate matrix tablets with silymarin as active ingredient. We used different Carbopols as matrix-forming polymers. The complexation of silymarin with different β -cyclodextrins was intended to increase the solubility of the active ingredient. By achieving sustained release, our aim was to offer a better alternative than the conventional unsuccessful oral therapy. Comparing the results of our research, we could select the Carbopol-cyclodextrin combination with the optimal drug release from the twenty different compounds. With these compositions, more than 85% of the silymarin was dissolved. The cytocompatibility of our product was proven, and the technology provided much better bioavailability than the conventional silymarin therapy.

The role of the gut microbiota in etiology of mental disorders

Vlatka Stojačić, Mirna Kovačević

Faculty of Medicine, J.J. Strossmayer University of Osijek

Introduction: Anxiety and depression are different mental and emotional disorders which have similar symptoms. Microbiome is a term for all microorganisms living in the human body, in this case it is the intestinal microbiome and bacteria that have a two-way communication with the brain. They work across different body systems.

Objective: Demonstrating a two-way relationship between the intestinal microbiome and the brain in anxiety and depression. Methods: It has been shown that many studies have been done to investigate the connection of microbial intestines with anxiety and depression disorders. The potential therapeutic and preventive effect of probiotics was found, the effect of a high fat diet on stress anxiety and cognitive dysfunction. Some of the studies have shown the effect of eating disorders and the effect of the mothers' obesity.

Results: In the research of a probiotic activity on pregnant and postpartum stage women, it was found that women who used probiotic significantly reduced depression and postpartum anxiety. Also, it has been shown that mothers high fat diets and obesity may hinder neurosion and affect behavioral disorders in childhood.

Conclusion: The impact of microbime on the brain is a relatively new topic in science. Although linking has been established, it is in the future to examine what are the mechanisms of action.

Key words: microbiome, intestine, brain, anxiety

Absorption of the ibuprofen and the appearance of its oxydative metabolits from small intestinal perfusate and the bile

Anett Szabo

Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Pécs

Introduction: After the per os administration of pharmacons, the molecules are absorbed from the gastrointestinal tract and reach the liver through the vena portae before they get into the sythemic blood circulation and finally to the site of action. Ibuprofen is typically metabolised and excreted as ibuprofen glucuronide, hydroxiibuprofen and carboxyibuprofen. Although in most cases its racemic form is administered, the (S)-(+)-ibuprofen has a higher biological activity.

Aims: In vivo investigation of intestinal absorption and metabolism of ibuprofen such as the appearance of its metabolites in the small intestinal perfusate and in the bile, under physiologic and pathologic (diabetic) conditions. Development of HPLC method for the identification quantification of ibuprofen and its main reported metabolits.

Methods: The experiments were performed on male Wistar rats. In urethan anaesthesia, an approximately 10 cm segment of the jejunal loop was isolated in vivo, and was perfused with an isotonic medium containing 250 μ M rac-ibuprofen. Another group of the animals was treated with streptozotocin (STZ) intravenously one week before the experiment. Samples were taken from the perfusion medium, and from the cannulated bile duct. From the perfusate the ibuprofen and its (R)- and (S)-enantiomers, from the bile the ibuprofen, and its main oxidative metabolites were measured.

Results: Among the ibuprofen enantiomers were taken in the end of the experiment the (S)-enantiomer was found a little bit higher amount in the perfusate. Excretion of the ibuprofen decreases in streptozotocin (STZ) induced hyperglycaemia, however, the quantify of the excreted oxidative metabolites significantly increased.

Conclusions: Presumably, higher amount of (S)-enantiomer is due to the active reexcretion into the intestinal lumen by the P-gp transporters, and the increased appearance of the oxidative metabolites can be explained by elevation of the nonenzyme-catalysed oxydation (oxidative stress) in hyperglycaemia.

Chemical analysis of essential oils using gas chromatography and their antibacterial activity with TLC-Direct Bioautography

Bettina Mariann Tigyi, Bela Kocsis, Gyorgyi Horvath

Department of Pharmacognosy, Faculty of Pharmacy, University of Pécs

Essential oils are very interesting natural products and among other qualities they possess various biological properties. The treatment of bacterial infections is presently problematic due to their antibiotic resistance. Therefore, the number of studies focusing on essential oils, as well as on their applications as new potential antimicrobial agents against human pathogenic microorganisms has recently increased. The aim of the present study was the chemical characterization of some traditionally used and therapeutically relevant essential oils (sweet fennel, spearmint, rosemary and cinnamon bark) and the optimized microbiological investigation of the effect of these oils on *Staphylococcus aureus*, *S. epidermidis*, methicillin-resistant *S. aureus* (MRSA), *Escherichia coli*, *Micrococcus luteus* and *Bacillus subtilis*. Determination of the chemical composition of the oils was performed by thin layer chromatography (TLC), gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS). The antibacterial effect of essential oils was investigated without separation and after separation of the components using a TLC-bioautographic method. Solvents with different composition were used as mobile phases. The antibacterial activity of sweet fennel, spearmint and cinnamon bark oils, as well as their main components (trans-anethole, carvone and trans-cinnamic aldehyde) were observed against all the bacterial strains used in this study. Undoubtedly, TLC-bioautography is a directly combined application of an analytical method with an in situ bioassay that facilitates a rapid identification of the active compound or compounds in a complex mixture. Further experiments focus on modelling the antibacterial effect of the essential oils involved in this study in a mouse airway inflammation model.

Biotechnology vs. small compound drug research. Advantages and limitations.

Karoly Tihanyi

Freelance-adviser, Keynote Speaker of Biotechnology and Health Sciences Session

In 2015 in Europe, out of the top-selling five products four were mAbs, chimera proteins or fusion proteins. The market share of biotechnology products keeps increasing worldwide. In 2008 the biotech sales amounted to 17 % of the total, while in 2017 it rised to 26 %. By 2022 some 29 % share is projected. However, the market share of biotech products seems to approach a plateau. The RnD spending of big pharma increases, while the relative spending, as a function of revenues reduced somewhat. Contrary, biotech firms' RnD spending is usually higher since they still develop their original products. While in generic small compound drug development the dissolution profile or a PK bioequivalence study provides ground for efficacy and safety, a biosimilar or a biobetter development usually requires a "quasy-original" development. The horrifying price of biologics is a strong motivation for new contestants in the market. Currently 8 biosimilar adalimumab are under development. Small compound drug research has been unproductive in certain indications for decades. Biologics often aimed at indications that are notoriously unsuccessful by small compounds. Legal and patent regulations related to biotechnology industry are still evolving and the question of interchangeability - related to the biosimilar or biobetter products - is still wide open. In the comparison of small compound drugs and biologics RnD, the research idea, preclinical development, safety issues, production, clinical investigation, upscaling of production, Investigators Brochure, authority approval and marketing all differ greatly.

Histological and Incidence Trends of Esophageal Cancer in a 26-year Period

Benedek Tinusz

Centre for Translational Medicine, Medical School, University of Pécs

Introduction: The most frequent histological subtypes of esophageal cancer are squamous cell carcinoma (SCC) and adenocarcinoma (AC). The incidence of AC has been increasing in developed countries. However, no data is available regarding the epidemiology of esophageal cancer in Hungary.

Aims: We aimed to evaluate the epidemiological trends of esophageal cancers in Hungary. Our hypothesis was that the incidence of AC is rising compared to SCC. **Methods** We conducted a case-control study in two centers of Pécs, Hungary, involving patients with esophageal cancer between 1992-2017. Data were collected on age, gender, histological subtype (as primary outcome) and localization of the tumor (as secondary outcome). Linear regression and chi-square tests were performed for the analysis.

Results: 666 patients were enrolled in the study. The relative incidence of AC and the age at the time of diagnosis significantly increased over the 26-year period ($p < 0,001$; $R = 0,726$ and $p < 0,005$; $R = 0,510$; respectively). There was no significant change in age at diagnosis in the AC group ($p = 0,768$; $R = 0,024$), however, we found a significant increase of age in the SCC group ($p < 0,001$; $R = 0,225$). In terms of localization, AC develops significantly more often in the lower and lower-middle part of the esophagus than in the upper and upper-middle part ($p < 0,001$).

Conclusion: The original Hungarian data analyzed acts as the novelty of this study. The increasing relative incidence of ACs and their predominantly distal location matches data in the literature. We suspect that the overall increase of age at diagnosis results from the combined effect of AC patients being older when diagnosed and the increasing age at diagnosis of SCCs. We plan to expand our current study to other centers as well as starting an esophageal cancer registry.

Interactions of mycotoxin metabolites 2'R-ochratoxin A and dihydrocitrinone with human serum albumin

Virag Voros, Zelma Faisal, Monika Balint, Csaba Hetenyi, Beata Lemli, Rita Csepregi,
Miklos Poor

Department of Pharmacology, Faculty of Pharmacy, University of Pécs

The nephrotoxic mycotoxins, ochratoxin A and citrinin, are common contaminants in foodstuffs. During the roasting process of ochratoxin A-contaminated coffee, 2'R-ochratoxin A (2'R-OTA) is formed; while dihydrocitrinone (DHC) is the dominant metabolite of citrinin. Human serum albumin (HSA) is the most abundant protein in the human circulation. HSA commonly plays an important role regarding the pharmacokinetics/toxicokinetics of several compounds. Both ochratoxin A and citrinin occupy Sudlow's Site I (subdomain IIA) as binding site on HSA.

In our study, stability of mycotoxin-HSA complexes and binding sites of 2'R-OTA and DHC on HSA were investigated. Binding constants were determined based on fluorescence spectroscopic methods, while binding sites were identified employing site markers and modeling studies. Finally, the influence of albumin on the acute toxicity of mycotoxins were tested on MDCK kidney cell line.

Both 2'R-OTA and DHC form stable complexes with HSA. 2'R-OTA binds with one magnitude lower affinity ($K=6.2 \times 10^6$ L/mol) to HSA than ochratoxin A, but binding site of 2'R-OTA is also Sudlow's Site I. Binding constant of DHC-HSA ($K=2.6 \times 10^5$ L/mol) and citrinin-HSA complexes are similar; however, binding site of DHC is located on subdomain IB (Heme binding site). Acute toxic effects of both mycotoxin metabolites were strongly alleviated by albumin, confirming their significant interactions with HSA.

In conclusion, complex formations of 2'R-OTA and DHC with HSA seem toxicologically relevant interactions. Since binding sites of these mycotoxins were identified, we can estimate which compounds may be able to displace them from serum albumin.

