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dedicated to the 50th anniversary

of the founding of Faculty of Pharmacy in Hradec Králové

Book of Abstracts

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YOUTH AND SPORTS

Wednesday 23. January 2019

Lecture hall A

Session 1 - Bioorganic and Pharmaceutical Chemistry: *13:00 – 17:00*

MICROBIAL RESISTANCE – ONE OF THE HOTTEST TOPICS OR THE PAST?!

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Microbial resistance is collocation that is declined all over the world. New bacterial strains that has developed resistance are noticed every year. Number of new cases are rising daily. There are rumours claiming that 10 million people will suffer and die of infectious disease caused by resistant strain in 2050. WHO has started few programs that should lead to elimination of resistance, *e.g.* END TB or Global action plan on antimicrobial resistance.[1,2] On the other hand, resistant microbes so called superbugs have their own mechanisms how to survive our attempts to kill them.

One way how to be succesfull in attempts to kill these microbes is to focus on finding novel antimicrobial compounds (new mechanism of action, advantageous pharmacological properties, broader spectrum of pathogens being influenced).

Design and synthesis of these compunds is crucial part of process but it is also very important to focus on biological properties such as minimal inhibition concentration, time-kill assays or mechanism of action (MoA) determination. MoA is also very important to understand the resistance mechanisms. We are able to determine MoA of potential antibiotic agent in four biochemical pathways – inhibition of cell wall synthesis, inhibition of DNA synthesis, inhibition of RNA synthesis or inhibition of proteosynthesis. This assay is based on the incorporation of radioactively labelled compounds, which are part of studied biochemical pathways. If detected radioactivity is lower, it means that potential antibiotic inhibits this pathway and radioactively labelled molecule cannot be incorporated into the final products. Standards used for this screening are vancomycin (inhibition of cell wall synthesis indicated by ^{3}H labelled *N*-acetylglucosamine), rifampicin (inhibition of RNA synthesis indicated by ^{3}H labelled uridine), ciprofloxacin (inhibition of DNA synthesis indicated by ^{3}H labelled thymidine), chloramphenicol (inhibition of proteosynthesis indicated by ^{3}H labelled leucine), and chlorhexidine (positive control, inhibition of all mentioned biosynthetic pathways).[3]

Currently, we are focusing on determining the possible MoA of potential antimycobacterial agents. We have revealed specific biomolecules that are connected with mycobacterial biosynthetic pathways. We have choosed ^{3}H labelled arabinose, which is involved in biosynthetic pathway of mycobacterial cell wall synthesis (ethambutol is used as standard – inhibition of arabinosyl transferase), and ^{3}H labelled acetic acid, which is involved in the same pathway (isoniazid is used as standard – inhibiton of mycolic acids synthesis). We need also to optimize MoA medium for culturing mycobacteria (*Mycobacterium smegmatis*, *M. aurum* and *M. tuberculosis* H37Ra), to set MIC for standards (rifampicin, isoniazid, ciprofloxacin, streptomycin, ethambutol, gentamicin, vancomycin, chlorhexidine) and to set time-kill properties.

The study was supported by Research program Development and Study of Drugs (Progres Q42).

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NEW UNALLOYED METAL NANOCONJUGATES AS CATALYSTS IN SELECTED REDUCTION OR ACETALIZATION REACTIONS FOR GREEN CHEMISTRY

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A series of mono, bi and tri-metal nanocatalytic transition metal systems were obtained and investigated. The catalysts included combinations of metals (Re, Ru, Rh, Ir, Pd) deposited on a silica support (SiO_2) or on a metal support (Ni or Mo). These are new, never described before, catalytic nanomaterials. The studied nanocatalytic systems may be reproducibly obtained by the described synthetic methods. These heterogeneous nanocatalysts can be used in industrial processes, especially in the reaction of carbon dioxide methanation, ammonia decomposition and glycerol acetalization.

In the low–temperature ammonia decomposition reaction, a high activity was obtained by the Pd_{NPs}/Ni catalyst which can be used to generate hydrogen in fuel cells. The Ru_{NPs}/Ni catalyst has a high activity in the reaction of low–temperature methanation of carbon oxides and can be used in methane production. Obtained nanocatalysts: Re/SiO_2, $ReRu/SiO_2$, $ReIr/SiO_2$, $ReRuIr/SiO_2$, $ReRhIr//SiO_2$, $ReRuRh/SiO_2$, $RuRhIr/SiO_2$, Ru/Mo, RuRh/Mo, RuRhIr/Mo and ReRhIr/Mo show high activity in acetalization reactions. Nano-Re supported on SiO_2 is a highly active and selective catalyst for the acetalization of glycerol into five-membered cyclic acetals (e.g., solketal) and can be used for the processing of glycerol waste. The tested catalytic systems may have practical application and serve for the development of the described industrial chemical processes, which may bring ecological and economic benefits.

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SUPRAMOLECULAR ASSEMBLY OF TETRAPYRAZINOPORPHYRAZINES INTO J-DIMERS

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Alkylamino substituted azaphthalocyanines (AzaPcs) have interesting spectral properties – they absorb in a wide range of UV-vis spectrum from 300 to 600 nm and quench fluorescence of other compounds. In non-coordinating solvents, these AzaPcs form J-dimers (Fig. 1) due to their planar aromatic core[1] that may affect their application as quenchers in oligodeoxynucleotide probes.[2] The tendency to aggregation can be driven by peripheral substitution. The goal of this project was to study the relation between peripheral substitution and stability of J-dimers (expressed as K_D). Therefore, a series of unsymmetrical AzaPcs (Fig. 2) was synthesized. Synthetic pathway included preparation of appropriate precursors, statistical cyclotetramerization, isolation of desired ABBB congener and introduction of zinc cation to the center of metal-free AzaPc. Stability of J-dimers was investigated in toluene by titration with pyridine. Obviously, increasing bulkiness of substituent caused destabilization of J-dimers.

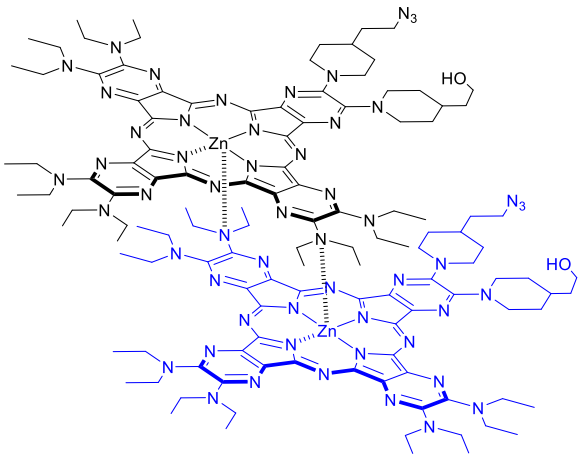

Fig. 1 - Example of AzaPc J-dimer

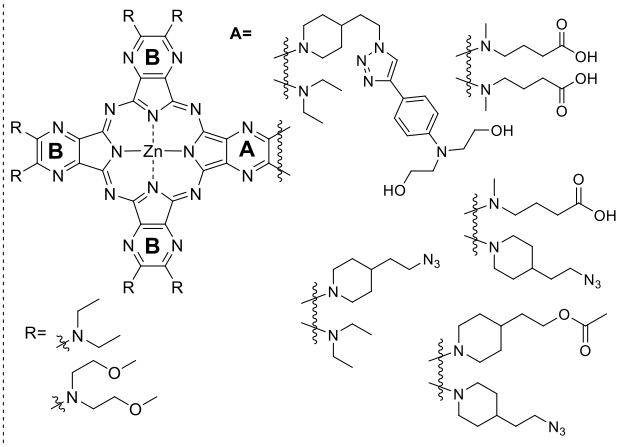

Fig. 2 - Structures of target unsymmetrical AzaPcs

The study was supported by Grant Agency of Charles University (1168217) and SVV 260 40.

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GOLD(I)-CATALYZED SYNTHESIS OF PIPERIDINE AMINALS AND THEIR FURTHER TRANSFORMATIONS

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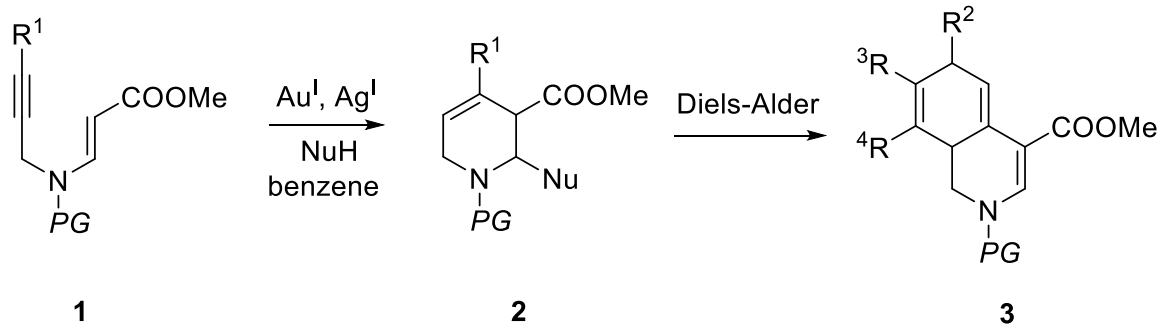
Synthesis of various types of heterocycles is possible from enyne precursors using cationic gold(I) species as catalysts. Our previous research on the cyclisation of propargyl vinyl ethers to dihydropyrans[1] as well as chemoselective cyclizations of β-propargylamino acrylic esters to dihydropyridines[2] was extended to include nucleophile-assisted reactions.

The optimized synthetic protocol was applied to the preparation of a library of substituted tetrahydropyridines **2**. Their further transformations via i.e. cycloadditions gave highly substituted isoquinoline derivatives **3**.The influence of structural factors on diastereoselectivity of cyclization as well as mechanistic considerations will be discussed.



1 **2** **3**

Scheme 1: Gold(I)-Catalyzed Synthesis of Piperidine Aminals

This work was supported by Charles University (SVV 260 401 and GAUK 262416) and Czech Science Foundation (Project No. 15-07332S).

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NARCOLEPSY AND COMPUTER-AIDED DRUG DESIGN APPROACHES

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Narcolepsy is a rare neurodegenerative disorder which is associated with decreased capacity to regulate sleep-wake cycles, excessive daytime sleepiness, cataplexy, vivid hallucinations and paralysis. This disorder is closely dependent on balanced activity of orexinergic system in the brain, involving interactions of specialized orexin neurons and peptide neuromediators (i.e. orexin A and orexin B). In the past decades, it has been discovered that narcolepsy could be treated by small molecule agonists of orexin receptor 2 (OX2R), the structure of which was determined by X-ray crystallography in 2015. OX2R is a trans-membrane receptor belonging to the group of G-protein-coupled receptors. Recently, novel drug candidates can be discovered using advanced computational technology such structure-based virtual screening (SBVS). In this project, a combined SBVS of roughly 1 000 000 chemical compounds (containing for example all clinically used drugs and several thousand of bioactive substances identified in traditional Chinese medicine drugs) was performed employing a cloud platform and a peta-flops-scale supercomputer to find potential drug candidates for activation of OX2R. 11 best ligands were tested *in vitro* to evaluate their agonistic activity towards OX2R. Actually, molecular dynamic simulations of these compounds complexed with OX2R are analyzed to reveal the molecular mechanism that determine the agonistic or antagonistic effect of a ligand. The simulations involve phospholipidic membrane (containing 1,2-dioleoyl-*sn*-glycero-3-phosphocholine, DOPC), a homologically optimized OX2R, the ligands, water and ions parameterized by a force field based on Gromos54a7. These complex computational studies have led so far to discovery of an antagonist of OX2R with IC_{50} = 2.2 μM.

The study was supported by Czech Science Foundation (Grant No. 17-08596S).

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THE STORY OF TACROXIMES; NOVEL UNIQUE COMPOUNDS FOR THE RECOVERY OF ORGANOPHOSPHORUS-INHIBITED ACETYLCHOLINESTERASE.

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The nerve agents, the most toxic chemical warfare agents, are known for over 70 years. Their deadly effect was demonstrated several times during the history. Another member of the organophosphorus compound family, organophosphorus pesticides, likewise represent serious burden for the mankind. Though, there is still no reliable antidote that would offer efficient medical assurance for the intoxicated patients. Herein, we describe two novel compounds, tacroximes, as unique merged molecules of tacrine against organophosphorous intoxication. These reactivators of acetylcholinesterase have balanced physic-chemical properties, should be able to cross blood brain barrier and have slightly lower cytotoxicity. Their efficiency was proved against dichlorvos as compared with pralidoxime and obidoxime. Tacroxime represents interesting starting point to spur the development of novel, centrally active reactivators/or prophylactic agents with potential to become interesting drug candidates for in vivo studies.

The study was supported by University of Defense (Faculty of Military Health Sciences, Long-term Development Plan and SV/FVZ201601).

SYNTHESIS OF PHTHALOCYANINE DERIVATIVES FOR SUPRAMOLECULAR INTERACTIONS WITH CUCURBITURIL

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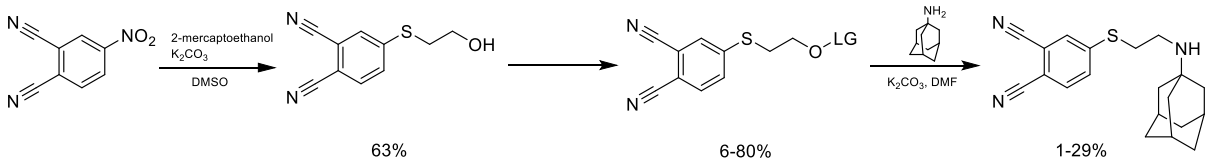
Phthalocyanines (Pc) are macrocyclic compounds structurally related to porphyrins, which are used e.g. as photosensitizers in photodynamic therapy in the treatment of tumor diseases. Their main disadvantage is their poor solubility in water and aggregation. Based on creating a supramolecular complex with cucurbiturils (CB) we can potentially improve those properties. CB are pumpkin-shaped macromolecules composed of methylene bridged glycoluril oligomers.[1] In this project we used one of the strongest reported supramolecular interactions between CB[7] and 1-aminoadamantane[2] as substituent on the Pc ring. We have successfully synthesized phthalocyanine precursor: adamantyl substituted phthalonitrile using reaction scheme bellow. Different synthetic approaches and optimization of this reaction will be discussed during the presentation. Binding of this phthalonitrile to CB[7] was studied using NMR in cooperation with colleagues from Masaryk University. Crystal structure of the complex was also obtained and confirmed the predicted orientation of adamantyl moiety in cavity of CB.



The study was supported by SVV 260 401.

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SYNTHESIS OF ELECTRONICALLY TUNED [3]DENDRALENES

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Dendralenes are acyclic cross-conjungated polyenes with an interesting, as yet unexamined reactivity and high potential for further synthesis.[1] We have focused on the synthesis of variously substituted [3]dendralenes containing electron withdrawing groups (e.g. carboxylic group), or a combination of electron withdrawing and donating groups. Synthesis is based on readily available *Z*-metallodienes 1, which are subjected to Migita-Stille coupling[2] yielding the intended final products 2 (Figure 1). Syntheses and possible applications of new compounds in domino Diels-Alder sequences and nucleophilic additions will be discussed.

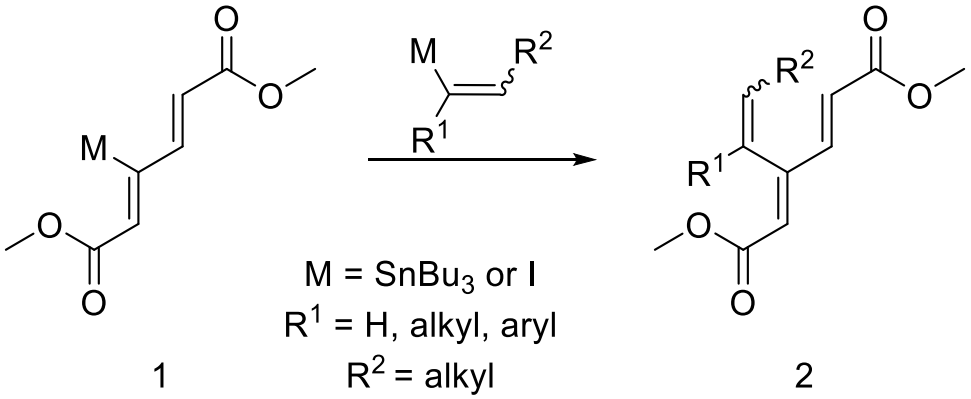

Figure 1

The study was supported by Charles University (SVV 260 401) and Czech Science Foundation (Project No. 18-17868S).

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DESIGN, SYNTHESIS, AND STRUCTURE ACTIVITY RELATIONSHIPS OF HYBRID COMPOUNDS COMBINING PYRAZINAMIDE AND P-AMINOSALICYLIC ACID AS POTENTIAL ANTIMYCOBACTERIALS

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Tuberculosis, or as it was known in Middle Ages “the white plaque”, has killed millions of people throughout history. This deadly infection was controlled with well-established drug regimen. Yet, the emergence of HIV and drug resistance have brought this old infection back to the list of the top ten causes of death worldwide.[1] Here we report the design, synthesis, and biological evaluation of a series of hybrid compounds combing pyrazinamide, which is a first line antitubercular drug, and *p*-aminosalicylic acid, which is a second line antitubercular drug, as demonstrated in the figure below. The compounds were obtained by reacting different pyrazinecarboxylic acids with *p*-aminosalicylic acid after being activated by 1,1'-carbonyldiimidazole in dimethylsulfoxide as a solvent. Obtained products were *in vitro* evaluated for their antimycobacterial activities against *Mycobacterium tuberculosis* H37Rv and four other non-tubercular mycobacterial strains. Furthermore, the compounds were *in vitro* screened for cytotoxicity against HepG2 liver cancer cell line. Most compounds exerted moderate to high activity against *Mycobacterium tuberculosis* H37Rv. Detailed results of biological evaluation and structure activity relationships will be discussed in the presentation.

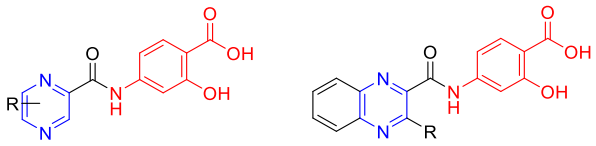

Fig.: The general structures of hybrid compounds combining pyrazinamide and p-aminosalicylic acid.

The study was supported by the Ministry of Education, Youth and Sports of the Czech Republic (SVV 260 401) and by Grant Agency of Charles University (project C-C3/1572317).

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HIGHLY SUBSTITUED PYRANONES VIA INTRAMOLECULAR TSUJI-TROST REACTION

BRŮŽA Z.[1]; KRATOCHVIL J.[1]; HARVEY J. N.[2]; RULÍŠEK L.[3]; NOVÁKOVÁ L.[1]; KUNEŠ J.[1]; KOČOVSKÝ P. [1,3,4];POUR M.[1]

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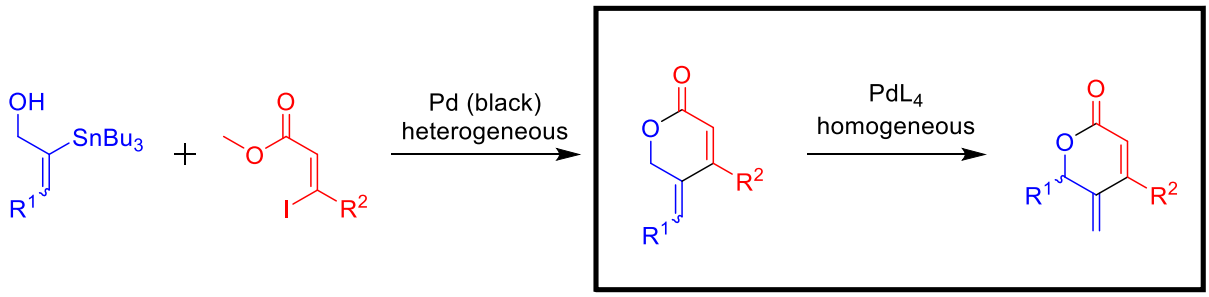
Nurturing minor reaction pathways into major transformations by implementing seemingly marginal changes of the reaction conditions may open interesting opportunities for obtaining new products from the same starting materials. To this end, we have developed an unusual isomerization of pyranones 1[1] (Scheme 1.) into 5-methylene pyranones 2[1]. The reaction is highly tolerant of a wide range of functional groups, and proceeds under mild conditions. Screening of chiral ligands was also performed to probe the possibility of enantiocontrol over the newly introduced chiral center. Additionally quantum chemistry calculations and kinetic simulations were performed to obtain insight into this unusual transformation.[2]



Scheme 1. General structures

The study was supported by Grant Agency of Charles University (project No. 1054216), Czech Science Foundation (project No. 18 17868S) and Charles University (SVV-260-401).

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SYNTHESIS OF SYMMETRICAL AND UNSYMMETRICAL ANIONIC PHTHALOCYANINES FOR PHOTODYNAMIC THERAPY

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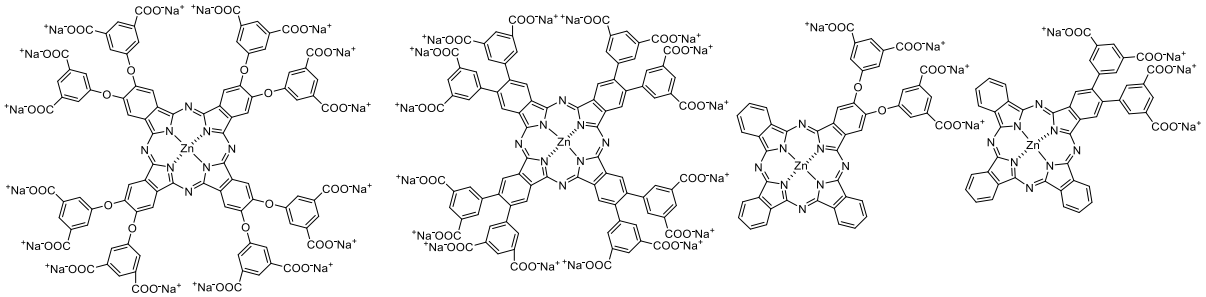
Phthalocyanines (Pcs) represent a promising group of organic dyes with interesting photophysical properties (strong absorption in area 650-750 nm and strong singlet oxygen production) highly suitable for the use in photodynamic therapy of cancer. The aim of this work was to synthesize symmetrical and unsymmetrical anionic Pcs with 3,5-dicarboxylatophenyl moiety connected with Pc core directly by C-C bond or by ether bridge. Unsymmetrical compounds with amphiphilic character can be incorporated to liposomes that may protect this phthalocyanine from binding to proteins or from aggregation at low pH. Precursors for synthesis of symmetrical and unsymmetrical Pcs, 4,5-disubstituted phthalonitriles, were obtained by Suzuki coupling or nucleophilic substitution with molecules bearing 3,5-dimethyl isophthalate. Symmetrical Pcs were obtained by cyclotetramerization reaction (initiator magnesium butoxide) of one precursor while unsymmetrical Pcs were prepared by statistical condensation of phthalonitrile with 4,5-disubstituted phthalonitrile. Methylesters were completely transesterified to butyl esters during this reaction. Magnesium complexes was converted to metal-free ligands and then to zinc complexes. Basic hydrolysis of ester bonds was the last step of the synthesis. Final Pcs were tested on photodynamic activity *in vitro* on HeLa cells. Photophysical properties and binding to serum protein of Pcs were studied.

The work was supported by GA UK 1060216 and SVV 260 401.



SYNTHESIS AND OPTIMIZATION OF THE SYNTHESIS OF 32-HYDROXYDOTRIACONTANOIC ACID

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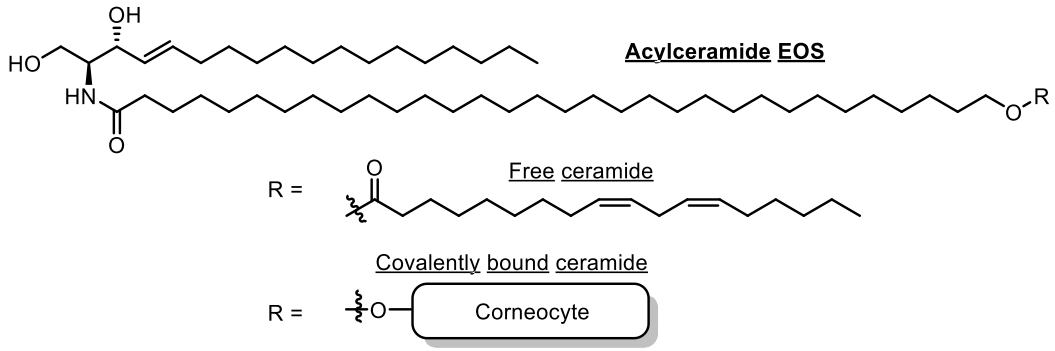
The 32-hydroxydotriacontanoic acid forms the backbone of all the acylceramides, which belong among ultralong chain ceramides. They are essential components of the *stratum corneum* and play a crucial role in proper function of the skin barrier.

The carboxyl group of this acid is bound to a primary amino group of the sphingoid bases and the ω-hydroxy group is either esterified with linoleic acid to form free ceramides or it can be linked to the surface of corneocytes in the form of covalently bound ceramides.



The recent literature describes the synthesis of 32-hydroxydotriacontanoic acid with relatively small yields. The most problematic part of the synthesis is the connection of two shorter fragments leading to the ultralong chain.[1] The main aim of this project is to prepare covalently bound ceramides on solid particles and to optimize the reaction conditions. The previously used Wittig reaction was changed for other olefinations, mainly Julia and Julia-Kocienski reactions and their modifications. The highest yields were so far obtained by the modified Julia-Kocienski reaction with (1-cyclohexyl-1*H*-tetrazol-5-yl) sulfonyl derivative of hexadecanoic acid as a starting material. In this case, we were able to increase the yield of this reaction even over 70 %, which greatly improved the described reaction pathway.

The study was supported by the Charles University (SVV 260 401) and by the Czech Science Foundation (16-25687J)

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INVESTIGATION OF THE COMPOUNDS INFLUENCING THE MELTING TEMPERATURE OF OLIGONUCLEOTIDE PROBES

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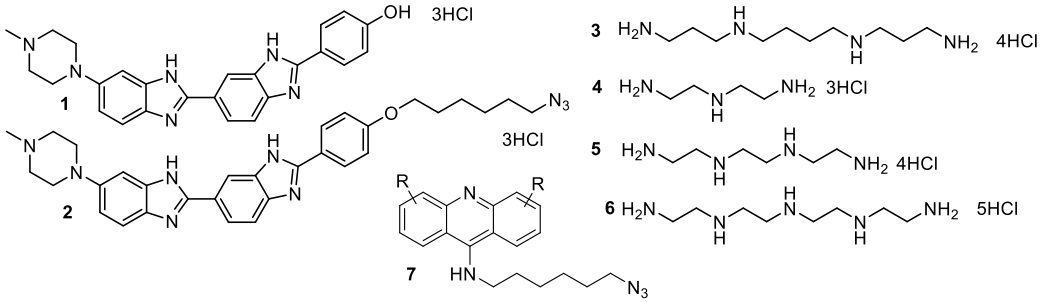
Real-time PCR is widely used method in various research fields. The use of longer probes is often not optimal for mismatch discrimination because of low melting temperature difference between fully complementary and mismatched probes. The use of shorter oligonucleotide probes can be advantageous in this case. On the other hand, low melting temperature of these shorter probes is major complication for practical use in real-time PCR. Minor groove binders (MGB) or intercalating dyes can stabilize the duplex and increase the melting temperature, e.g. biogenic polyamines have strong interaction with DNA and RNA.[1] For testing the capability to increase melting temperature, several compounds were selected. Commonly known MGB Hoechst 33258 (**1**),[2] its modified derivative **2**, naturally occurring spermine **3**, three artificial polyamines (**4**-**6**) and 14 acridine derivatives (**7**) were tested for their capability to increase melting temperature of shorter probes. Modified Hoechst 33258 (**2**) and 14 acridine derivatives (**7**) were prepared in our laboratory. The tests revealed interesting increase of the melting point of the probes for several compounds.



The study was supported by Technology Agency of the Czech Republic (TH03010251) and The Charles University Grant Agency (994218)

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STABILITY EVALUATION OF MAGNESIUM COMPLEXES OF PHTHALOCYANINES AND AZAPHTHALOCYANINES UNDER ACIDIC CONDITIONS

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Phthalocyanines (Pc) and their azaanaloges (AzaPc) represent macrocyclic compounds with a large system of conjugated bonds. Due to this system, they possess interesting photophysical and photochemical properties such as strong emission of fluorescence or production of singlet oxygen, which make them widely examined as potential diagnostic and therapeutic agents.[1] Importantly, the central cation incorporated in the macrocycle changes the relaxation pathways of the excited states on the basis of heavy-atom effect.[2] Magnesium is the lightest stable central cation and for this reason its complexes are characterized by strong fluorescence; advantageously used as fluorescent probes or labels.[1] However, the instability of magnesium complexes in acidic environment (demetallation to metal-free derivatives) is the main obstacle of wider utilization of these fluorescent dyes. Once a macrocycle is demetallated, it loses its strong fluorescent properties. In this work, we decided to more closely evaluate a demetallation and other decomposition of these compounds in water solution at five different pH ranging 1 – 7.4. The stability was monitored by absorption spectroscopy for 24 h where characteristic splitting of the Q-band occurred after demetallation. Experiments proved that the more acidic environment, the faster process of the demetallation occurs and that Pcs are less stable than corresponding AzaPcs. We also tested possible protection provided by various delivery systems (liposomes and microemulsions). In particular liposomes showed high level protection where no changes in absorption spectra of AzaPc was detected after 24 h even at the most acidic pH 1.

The study was supported by Charles University (reg. No. SVV 260401).

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Wednesday 23. January 2019

Lecture hall B

Session 2 - Clinical and Social Pharmacy: *13:00 – 14:45*

DEVELOPMENT AND IMPLEMENTATION OF INTERVENTIONS TO PROMOTE MEDICATION ADHERENCE IN KIDNEY TRANSPLANT PATIENTS

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Adherence to complex therapeutic regimen is essential during the whole posttransplant period. Nevertheless, it decreases over time and changes according to patient's beliefs and regular care at the clinic (1). The aim is to introduce the design of our study developing and implementing individual multicomponent interventions for supporting medication adherence in patients after kidney transplantation and evaluating their impact on behavioral and transplant outcomes. This single-centre, prospective, interventional study will be undertaken in the University Hospital Hradec Kralove in 2019-20, where all adults on basal immunosuppression (IS) will be approached to mitigate selection bias. Patients at risk of non-adherence to IS will be identified, using laboratory (medication level variability index) as well as self-report (BAASIS® questionnaire) measurement of medication adherence, combined with reports by family member or caregivers. These patients will be invited to strucuted interview with a pharmacist and will receive tailored set of interventions based on their individual barriers in knowledge, medication taking routine or attitudes. Interventions will be developed based on previous research results as well as the needs of the health care facility reflecting all main causes of non-adherence to IS. Study will include the baseline visit and the follow-up after 6 and 12 months. Effectiveness of interventions will be tested on both behavioral (e.g. adherence to IS and self-management, beliefs and knowledge about the treatment) and transplant outcomes (e.g. progression of graft dysfunction, rejection episodes, graft loss). If interventions proof to be effective, they can be implemented into standard posttransplant care and serve as the template for other clinics.

The study was supported by Charles University (Project SVV 260 417).

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NUTRITIONAL INTAKE OF ENERGY AND SUBSTRATES, THEIR CHANGES IN PREGNANT WOMEN DURING THE LAST DECADE

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Malnutrition of the maternal organism during intrauterine fetal development may result in disease in the next life of an individual. The aim of the study was to evaluate the changes in the intake of nutritional energy and substrates (PNES) in Czech pregnant women over the last 10 years and determine the accuracy of the predictive equations for PNES.[1] Thirthy-five Czech pregnant women 29±2,79 years old were attended in the pilot study. PNES in individual trimesters of pregnancy was obtained in weekly nutritional records, then evaluated by the computer program NutriDan and compared with values from the predictive equations for PNES.[1] In 10 years, PNES (according to the predictive equations) was increased in protein in the 1st trimester of pregnancy by 9,58% (p = 0,02) and decreased in carbohydrate intakes in all pregnancy periods by 10,06% (p = 0,04); by 14,60%. (p = 0,0002); by 12,71% (p = 0,00003). Differences in PNES on weekdays were does not change despite the expectation. The predictive equations for PNES can be used during pregnancy even after 10 years, except for protein intake in the 1st trimester and carbohydrates throughout pregnancy.

The study was supported by GA UK č. 1306218, SVV/2017/260417, MH CZ –DRO (UHHK, 00179906) a PROGRES Q42

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NO FAULT VACCINE COMPENSATION PROGRAM OVERDUE IN CZECH REPUBLIC - SERIOUS THREAT FOR THE CZECH MEDICAL SYSTEM IN A PROSPECT OF THE NEW CIVIL CODE.

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On the first of January 2014 the New Civil Code (Law 89/2012 Coll., Civil Code) has come into the force in the Czech Republic. It has changed many aspects of everyday life, including the problem of liability following adverse events attributed to the no-fault administration of compulsory vaccines. Until then the proprietor of health service facility was held strictly liable, for any damages that aroused from the application of vaccine even if it was caused by the nature of the applied vaccine (§421a of Law 40/1964 Coll., Civil Code). After the new legislation come into force this is no longer true. Since 2014 the Health service provider should be held liable only, if there is his negligence proved. Thus question come into mind – who is responsible for such adverse effect now.

The position of the Czech government is unclear but it steers towards the manufacturers' liability. We think that this is the moral hazard. In our opinion the manufacturer should not be held liable, because he openly states in the SPC that such effects, even rare, could occur. It is the government that demands mandatory vaccination with penalization to offender for those that not conform, so it follows that government should be responsible for any adverse effects caused by such vaccination. Majority of countries united in the OECD have adopted some form of a compensation for individuals harmed by compulsory vaccines administration. In this presentation I would try to evaluate such programs with emphasis on the states of comparable size, similar legal systems and comparable level of public health systems and make proposition for such a program tailored for needs of the Czech Health care system.

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SPONTANEOUS ADVERSE DRUG REACTIONS REPORTING TO ORAL ANTICOAGULANTS IN THE CZECH REPUBLIC

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Shorter clinical use of direct oral anticoagulants (DOACs) leads to need for yet unknown risks monitoring. The aim of the study was the analysis of spontaneous adverse drug reactions (ADRs) reports to oral anticoagulants (OACs) in the Czech Republic.

Retrospective analysis of spontaneous reports of suspected ADRs to OACs from healthcare professionals and patients was conducted. Reports to warfarin, dabigatran, rivaroxaban and apixaban between 01/2005–11/2017 were identified in the State Institute for Drug Control (SUKL) database and quantified as anonymized data in MS Excel 2019. The reports were evaluated by ADR character, seriousness, and consequence, and patient´s characteristics. The incidence of ADRs was calculated per exposure units expressed as million defined daily doses (mDDD). Drug utilization data come from the SUKL database of quarterly reported drug supplies from distributors to healthcare facilities. Descriptive statistics and disproportionality analysis (DA), using reporting odds ratio (ROR) and 99% confidence interval (CI) was performed.

SUKL received 297 reports to OACs with 672 ADRs. DOACs were related to 65% reports, resp. 64% ADRs. The most frequent ADRs were hemorrhagic and thromboembolic events, gastrointestinal and skin disorders. The DA showed that number of DOACs´ ADRs exceeded warfarin (ROR=10.77, 99% CI=8.70–13.32) and number of ADRs of dabigatran exceeded rivaroxaban (ROR=3.90, 99% CI=2.88–5.29). In 96% of reports serious ADRs were noticed, of which 21 were fatal. In context with higher warfarin utilization, the ratio of ADR reports and mDDD was low; conversely to DOACs. In summary, the number of reports seems to be low, but most ADRs were serious. Higher reporting to DOAC could be a result of shorter time on the market, compared to warfarin long-term use and reduced susceptibility to the ADRs[1].

The study was supported by the Charles university grant (SVV 260 417).

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FACTORS INFLUENCING ANTIBIOTIC PROPHYLAXIS FOR SURGICAL SITE INFECTION PREVENTION

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Antibiotic prophylaxis (AP) plays an important role in a reduction of a surgical site infection (SSI). The aim of the presented study is to verify the use of the risk index as one of the tools for the individualization of the patient's AP in clinical practice. In February 2018, we conducted a cross-sectional study, in which we identified total length of AP as the main problematic area. Special tool – the Risk Index could be used as the basis for the indication and the total length of AP among the common principles (dirty-infected wound, the presence of foreign material, pharmacokinetics of antibiotic etc.). Before a surgical procedure, we could calculate the ACS (American College of Surgeons) Risk Index, which comprises risk factors (RFs), physical status and the type of surgery. ACS Risk Index estimates the chance of an unfavorable outcome such as SSI or the postoperative complications. After the surgical procedure, the NHSN (National Healthcare Safety Network) Risk Index could be calculated according to the ASA score (classification system that assesses the physical status of patient before surgery into 5 classes), the duration of operation and the wound classification. In 2019, we plan to perform cross-sectional study with an approximate number of 300 patients. In addition to the systematic literature review, we will collect patient and surgical procedure data before, during and after surgery. An AP individualization will be performed. Usability of ACS Risk Index and NHSN Risk Index will be evaluated with the postoperative complication analysis. These will be monitored 30 days after the surgery if no foreign material being used or 1 year if patient obtains foreign material during the surgical procedure. Regarding these results, the interventions will be arranged to optimize and individualize use of AP.

The study was supported by Charles University (Project SVV 260 417).

ANALYSIS AND MANAGEMENT OF NURSES' MEDICATION ERRORS DURING DRUG ADMINISTRATION

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During hospitalization, there is a frequent occurrence of various mistakes caused both by healthcare professionals and by patients. A major part of these comprises medication errors during inappropriate drug handling. The key role is played by the possibility of preventing these errors. The aim of the study is to analyse medication errors by direct observation of the nurses during drug preparation and administration.

The observation was conducted prospectively during 12 days in an inpatient rehabilitation facility. Three trained pharmacists monitored morning, noon and evening drug administration processes on seven wards. All errors were documented in real time, according to the predefined forms. The form always contained the name, strength, pharmaceutical form and dosage of all drugs prescribed to one patient. Each form was created for each patient individually, based on his or her regular medication. Within the form, the observed errors were further classified into 34 categories, including e.g. storage and labelling of the drug, administration route or drug strength. The obtained data were digitized and analysed.

Nurses (*n=27*) administered in total 4662 individual doses, on average 8.18 (SD ± 5.19) doses per patient day (*n=570*). Of these doses, 92.8 % were administered orally, 5.1 % subcutaneously, 1.6 % topically, and 0.5 % other. Severe misconducts (drug name, or drug strength confusion, and drug omission) occurred in 21 (0.45 %) cases, on average 0.037 error per patient day. Patients hospitalized for more than 20 days, are more than 50 % likely to experience severe nurses' medication error.

The analysis of the observed errors has revealed that nurses commit severe and less severe mistakes, which ultimately can lead to patient harm. It is therefore necessary to focus on their minimization.

The study was supported by Charles University grant (SVV 260 417).

ANALYSIS OF PHARMACOTHERAPY AS A ONE OF THE MAIN FALL-RELATED RISK FACTOR – A 12-MONTH PROSPECTIVE STUDY IN HOSPITALS OF SOUTH BOHEMIA REGION

VOSÁTKA, J.,[1] MALÝ, J.,[1] DOSEDĚL, M., [1] BRABCOVÁ, I., [2] HAJDUCHOVÁ, H., [2] BÁRTLOVÁ, S.,[2] KUBĚNA, A.,[1] KOLÁŘ, J.,[1] VLČEK, J.,[1] TÓTHOVÁ, V.,[2]

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Pharmacotherapy belongs among one of the modifiable and predictive risk factors of falls. The aim of the study was to analyse the effect of pharmacotherapy and drug-related factors on falls at hospitalized patients with fall during 2017 at 16 hospital wards of four hospitals in South Bohemia region.

The results of this prospective study were collected through online instrument containing data about patients with falls. The data obtained from patient´s medical records (e.g. drug and personal anamnesis, selected laboratory results) were completed with other information (e.g. circumstances of fall, internal and external risk factors). The data analysis was primarily focused on drugs, diagnoses and associated risk factors increasing risk of falls. Potential and individual risks were determined for each patient who experienced fall. The potential risk represented all drugs that showed an increased risk of falls described in current literature or according to its mechanism of action. If a clinical pharmacist could not exclude the drug influence on fall probability, the drug was reported as with individual risk. For data analysis, the descriptive and analytical methods were used. A p-value <0.05 was considered statistically significant.

280 falls (51.1% women; mean age 77 ± 12), mean 8.8 drugs, and 4.1 drugs with potential or 1.8 individual risk per fall were identified. Drugs affecting the cardiovascular, or the central nervous system demonstrated the highest potential risk (>60%). Use of potential risk drugs were positively associated with increasing age ($p = 0.007$).

The application of pharmacotherapy-related predictive risk factors together with individual approach to the patient can be an effective preventive strategy in drug-induced falls.

The study was supported by a grant of Charles University (SVV 260 417). Supported by Ministry of Health of the Czech Republic, grant nr. 16-33463A.

Wednesday 23. January 2019

Lecture hall B

Session 3 - Pharmacognosy and Toxicology of Natural Compounds: *15:00 – 17:30*

AMARYLLIDACEAE ALKALOIDS FROM *NARCISSUS PSEUDONARCISSUS* CV. DUTCH MASTER AS POTENTIAL DRUGS IN TREATMENT OF ALZHEIMER´S DISEASE

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Alzheimer´s disease (AD) is the most prevalent neurodegenerative disease worldwide with complex etiology and multifaceted pathophysiology. AD is characterized by massive deposits of amyloid-β peptide, neurofibrillary tangles of the hyperphosphorylated τ-protein and inflammatory mediators leading to neuronal death. AD is manifested by damage of cognitive and noncognitive functions[1].

The genus Narcissus from the Amaryllidaceae family is mainly distributed in south-western Europe, North Africa. This family contains special type of alkaloids (AA), possessing a wide range of pharmacological properties such as antitumor, antiviral and acetylcholinesterase (AChE) inhibitory activity. AA galanthamine is used for AD therapy[2].

Twenty-two Amaryllidaceae alkaloids of various structural types have been isolated. The bulbs were processed by extraction, followed by column and preparative chromatography and recrystallization. The chemical structures were elucidated by combination of MS, HRMS, NMR spectroscopic techniques. All isolated compounds were evaluated for their in vitro AChE, butyrylcholinesterase (BuChE), prolyl oligopeptidase (POP) and glycogen synthase kinase-3β (GSK-3β) inhibitory activities. The most important biological profile has been demonstrated by narcimatuline ($IC_{50,BuChE}$ = 5.9±0.2 μM, $IC_{50,POP}$ = 29.2 ± 0.9 μM; $IC_{50,GSK-3\beta}$ = 20.8 ± 2.4 μM).

Acknowledgements: This project was supported by Charles University grants (SVV UK 260 412; 260 401; Progres/UK Q40 and Q42).

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BRUNSVIGINE ISOMER AS A POTENTIAL AGENT IN THE TREATMENT OF ONCOLOGICAL DISEASES

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Plants from Amaryllidaceae family are interesting source of specific bioactive compounds - Amaryllidaceae alkaloids (AmA). So far, nearly 600 AmA of various structural types have been detected. Among the most important biological activities of AmA belong activities associated with Alzheimer's disease (AD) and the antineoplastic activity. These and other diseases of affluence are becoming increasingly widespread all over the world. From this reason the development of new potential drugs is needed. Galanthamin is already used as a reversible selective inhibitor of human erythrocytic acetylcholinesterase (HuAChE; $IC_{50\ HuAChE} = 1.5 \pm 0.2\ \mu M$)[1] in patients with AD. Many AmA have been screened for their activity to inhibit the growth of different cancer cell lines and active compounds can be also used as lead structures for a preparation of their semisynthetic analogues (mainly lycorine and haemanthamine).

From *Narcissus* cv. PROFESSOR EINSTEIN summary alkaloidal extract 25 different alkaloids have been isolated so far and they were identified by MS, HRMS and 1D- and 2D-NMR techniques and X-ray. All alkaloids were tested on their activities associated with AD (AChE, BuChE, POP, GSK-3β) and their ability to inhibit the growth of several cancer cell lines. From isolated alkaloids, the newly isolated isomer of brunsvigine gave the best results in the screening. IC_{50} determination was done ($IC_{50\ A549} = 2.29 \pm 0.43\ \mu M$, $IC_{50\ SAOS\text{-}2} = 2.20 \pm 0.25\ \mu M$) and now it has been undergoing determining of the site of interference in the cell cycle.

The study was supported by SVV 260 292 and SVV 260 412.

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PAPAVER RHOEAS: THE SOURCE FOR NMR ELUCIDATION

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The presented alkaloids were isolated from *Papaver rhoeas* (Papaveraceae) at the Department of Pharmaceutical Botany, Faculty of Pharmacy, Hradec Králové. The Papaveraceae family is very rich in specific alkaloids derived mostly from isoquinoline.

The crude alkaloid extract displayed a promising inhibitory effect on butyrylcholinesterase. As such, it was further subjected to isolation and structural analysis of its constituents.

The isolated substances were characterized and interpreted by employing standard ^{1}H, ^{13}C, gCOSY, gHSQC, gHMBCAD and NOESY experiments on a Varian VNMR S500 spectrometer, supported by EI-MS spectra.

Each of the isolated alkaloids was later screened for biological activities on acetylcholinesterase, butyrylcholinesterase and prolyloligopeptidase. Selected compounds were also tested for cytotoxicity.

The study was supported by Czech Science Foundation (project GA ČR 18-17868S) and Charles University (project SVV 260 401).

ONE-STEP ISOLATION OF LUTEIN FROM GREEN MICROALGAE (*CHLORELLA VULGARIS*) BY HIGH PERFORMANCE COUNTERCURRENT CHROMATOGRAPHY

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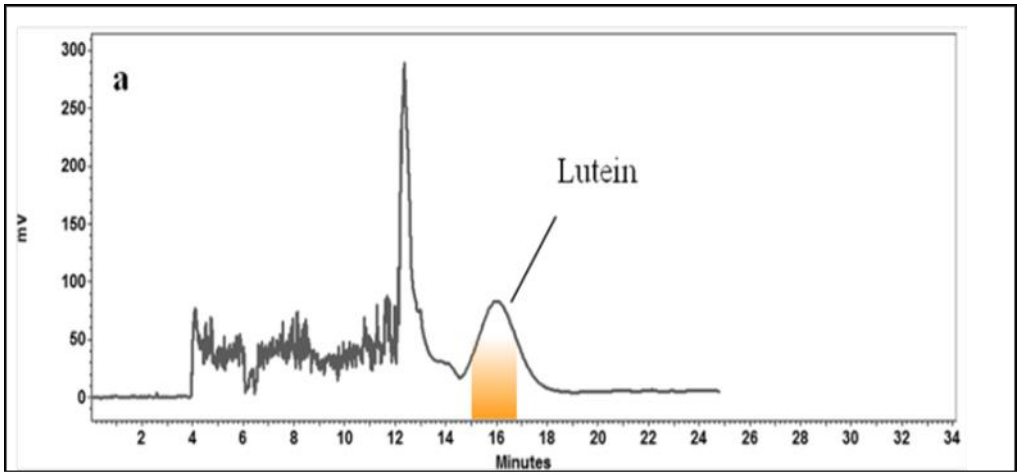
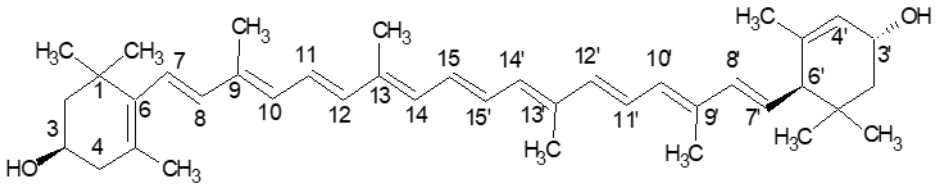
Lutein is a yellow carotenoid that naturally occurs in green leafy vegetables, orange-yellow fruits, and flowers. It is an antioxidant compound with eye health promoting properties and its utilization as an active ingredient in dietary supplements is currently driving the growing industrial demand for this compound. The flower petals of yellow Marigold (*Tagetes erecta* L.) represent the most important commercial source of lutein; however, their utilization is limited by seasons, climate, planting area, and the high labor costs.[1,2] Green *Chlorella vulgaris*, an eukaryotic microalga, has recently become a promising alternative feedstock for lutein. So far, the commercial lutein is mainly obtained from Marigold flowers by solvent extraction, but this procedure has a limited specificity to the target compound. Therefore, the application of an efficient and scalable isolation technique is pivotal for obtaining high-quality commercial lutein. In the present study, a high-performance countercurrent chromatography (HPCCC) method was developed and applied to obtain lutein from *C. vulgaris* biomass. Different two-phase solvent systems composed of *n*-heptane, ethanol and water were evaluated for their capacity to provide a proper distribution coefficient ($0.5 \leq K \leq 2.5$) of lutein and for exhibiting both an adequate density difference between the two phases (≥ 0.080 g mL^{-1}) and a short settling time (< 30 s). The two-phase solvent system composed of *n*-heptane–ethanol–water (5:4:1.5, v/v/v) was selected for the isolation of lutein. In addition, different flow rates (1–8 mL min^{-1}) and sample loadings (200–400 mg of extract) were examined to optimize the HPCCC separation conditions. Under the optimized operating conditions, the lower phase of the selected solvent system was used as the mobile phase at a flow rate of 8 mL min^{-1}, whereas the rotational speed and temperature of the separation column were 1200 rpm and 28 °C, respectively. The retention of the stationary phase at the end of the HPCCC separation was 52%. Overall, 10 mg of lutein with purity of 97% was obtained from 200 mg of *C. vulgaris* extract. The chemical identity of the target compound was confirmed by high performance liquid chromatography with UV-visible detection (HPLC-DAD) in comparison with an authentic standard. The method described in this study represents a good strategy to efficiently isolate lutein from microalgae biomass and can serve as a good reference to scale up the production of this bioactive compound at pilot and industrial size.

Figure 1. Chromatogram of the HPCCC isolation of lutein from microalgae *Chlorella vulgaris.*



Lutein



Acknowledgements: This study was supported by Charles University Grant´s Agency (*GAUK 1134217*) and the *SVV 260294, TAČR TJ01000013* grants. T. Fábryová gratefully acknowledges the scientific supervision of Dr. Cheel in Algatech-Czech Academy of Sciences.

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IMMUNOMODULATORY ACTIVITY OF SCUTELLARIA BAICALENSIS AND AZORELLA COMPACTA

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Infectious diseases are an ever-present threat that can be potentially deadly. A negative role is also accounted to the gradually increasing resistance of microorganisms to antibacterial medicines. Enhancing the immunity against infectious agents is, therefore, important goal of searching for new resources of active substances. These could be found in *Scutellaria baicalensis* L. (Baical skullcap, family *Lamiaceae*), one of the medicinal herbs with a long history of usage in traditional Chinese medicine. Immunomodulatory activity of *S. baicalensis* has already been proven to some extent[1], but it is still unknown which kind of extract (ethanolic or aqueous) and in which concentration is more effective and which content substances have the highest share on this activity. Another possible source of immunomodulatory active substances is *Azorella compacta* Phil. (syn. *A. yareta*, Llareta, family *Apiaceae*), a cushion shrub grown at altitudes of the Andes in South America's puna. Experiments with aqueous extracts proved the antioxidant and immunomodulatory effect of contained polyphenols[2], but the effect of the ethanolic extract on immune cells is still unknown. In the presented study, the immunomodulatory activity of extracts from *Scutellaria baicalensis* and *Azorella compacta* was investigated through CD69 antigen activation, together with seven main flavonoids from *Scutellaria*. In addition, the content of three important flavonoids in Bacial skullcap (baicalin, baicalein, wogonoside) was evaluated as well.

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ALKALOID PROFILING OF HIPPEASTRUM CULTIVARS BY GC-MS, ISOLATION OF AMARYLLIDACEAE ALKALOIDS, AND EVALUATION FOR THEIR CYTOTOXIC ACTIVITY

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Six alkaloidal extract of ornamental varieties of *Hippeastrum* have been studied for their alkaloid profile by GC/MS. Twenty-one compounds with typical mass spectra of Amaryllidaceae alkaloids (AA) were detected. Nineteen of them were identified based on their mass spectra, retention times and retention indexes. Identified alkaloids belong to the crinine, haemanthamine, galanthamine, homolycorine, lycorine, montanine, and tazettine structural type of AA.

Using preparative TLC, five Amaryllidaceae alkaloids have been isolated in pure form from various *Hippeastrum* cultivars. The compounds were identified by MS, 1D and 2D NMR spectroscopic analyses and by comparison of the obtained data with the literature as montanine (**1**), vittatine (**2**), 11-hydroxyvittatine (**3**), lycorine (**4**) and hippeastrine (**5**).

Three of the isolated compounds (montanine, vittatine and hippeastrine) have been screened on a panel of human cancer cells (Jurkat, MOLT-4, A549, HT-29, PANC-1, A2780, HeLa, MCF-7 and SAOS-2) fot their in vitro cytotoxic activity. In this work, montanine (**1**) has been found to display strong cytotoxicity against all tested cancer cell lines [1]. This compound has been selected for determination of IC_{50} values.

The study was supported by SVV 260 412 project.

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ISOLATION OF AMARYLLIDACEAE ALKALOIDS FROM *ZEPHYRANTHES CITRINA*

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Zephyranthes is a genus of bulbous perennial plants belonging to family Amaryllidaceae. The plants of this family are used by native people of different countries for treating various diseases. The genus *Zephyranthes* consists of about 90 species and only some of them have been studied for their chemical constituents. The phytochemical work on this genus revealed the diversity of compounds especially alkaloids having various pharmacological activities as anticancer, anticholinesterase and antiviral, antifungal and antiinflammatory. To date, ten alkaloids of various structural types have been isolated from *Zephyranthes citrina*[1].

The summary ethanolic extract was prepared from the fresh bulbs of *Zephyranthes citrina* (30 kg) and separated on column chromatography. More than six hundred fractions were collected, and pooled together based on TLC into 21 subractions. So far, twenty-one alkaloids in pure form have been isolated. The isolated compounds were identified by comparison of obtained analytical data (MS, NMR, optical rotatory) with the literature data. All isolated alkaloids were assayed for their biological activities connected to Alzheimer´s disease (inhibition of cholinesterases, GSK-3β, ability to permeate through the blood-brain barrier), and anticancer potential (cytotoxicity agains panel of cancerous and noncancerous cell lines).

The study was supported by Charles University grant GA UK 178518 and SVV 260 412 project.

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DERIVATIVES OF AMARYLLIDACEAE ALKALOIDS ISOLATED FROM *NARCISSUS* CV. CARLTON AND THEIR BIOLOGICAL ACTIVITY

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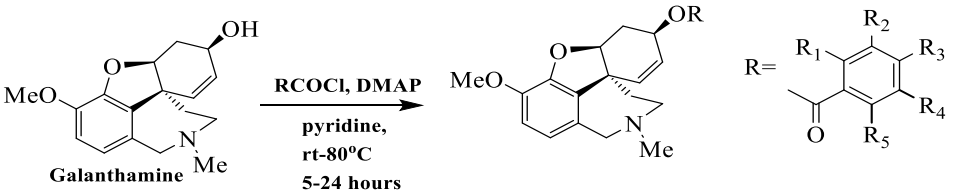
Plant of family Amaryllidaceae comprises about 85 genera and 1100 species that distributed widely in tropical and subtropical region of the world. *Narcissus* cv. Carlton is an interesting species with high content of Amaryllidaceae alkaloids (AA), which belongs to this family. More than hundred alkaloid has been isolated from *Narcissus* genus. AA are classified into nine skeleton types e.g. norbelladine-, lycorine-, homolycorine-, crinine-, haemanthamine-, narciclasine-, tazettine-, montanine- and galanthamine-. *Narcissus* alkaloids have remarkable therapeutic application as antitumor, antifungal, antimalarial, antibacterial, acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) inhibitory activity[1]. Galanthamine is one of the most abundant AA and clinically used for the treatment of Alzheimer's diseases as reversible inhibitors of AChE. Different derivatives of AA are reported to display wide range of biological activities e.g. anticancer, antimicrobial, antimalarial, antioxidant, AChE and BuChE inhibitory activity. Semi-synthetic derivatives of galanthamine display potential AChE inhibitory activity, antimicrobial and antioxidant activity. However, anticancer properties of various galanthamine derivatives have not studied yet. The aim of this study is to prepare aromatic esters of galanthamine derivatives (substitutions in different positions on aromatic ring – methyl groups, methoxy groups, nitro groups and hologens). So far, sixteen semi-synthetic derivatives of galanthamine have been prepared and their structure has confirmed by NMR and MS methods. All derivatives undergo different biological tests connected with Alzheimer's and oncological diseases.



This project was supported by Charles University grant SVV UK 260 412.

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SEMISYNTHETIC DERIVATIVES OF AMARYLLIDACEAE ALKALOID HAEMANTHAMINE AS POTENTIAL DRUGS IN THE TREATMENT OF ALZHEIMER'S DISEASE

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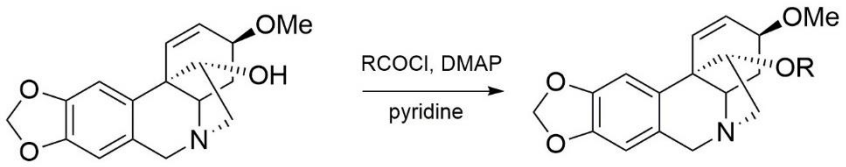
Plants of the Amaryllidaceae family contain approximately 85 genera and 1100 species, have a wide distribution through both tropical and sub-tropical regions worldwide. They have also a long and notable place in the history of traditional and Western medicine. Alzheimer's disease (AD) is the most prevalent neurodegenerative disease worldwide with complex etiology and multifaceted pathophysiology and data indicate an exponential rise in the number of cases of this disease. The well-known Amaryllidaceae alkaloid (AA) galanthamine is marketed drug for AD therapy under the commercial name Reminyl© (galanthamine hydrobromide). Other alkaloids like haemanthamine (HA) and lycorine have demonstrated interesting antitumor and/or apoptotic effects and other studies also pointed out various pharmacological properties of semisynthetic derivatives of some Amaryllidaceae alkaloids.

One of the most interesting AA alkaloids is alkaloid haemanthamin, which is widely distributed through Amaryllidaceae plants. Based on our previous results, where we reported promising anti-cholinesterase activity of pilot series of HA derivatives, we decided to continue in preparation of further semisynthetic derivatives. [1]

So far, we prepared four new aromatic esters of HA containing different substitutions on aromatic ring. New compounds are identified by 1D and 2D NMR, GC/MS and ESI-MS methods, and all substances are screened for different biological activities connected with potential treatment of AD.



This project was supported by Charles University grants (SVV UK 260 412)

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DERIVATIVES OF AMARYLIDACEAE ALKALOIDS OF MONTANINE TYPE AND THEIR BIOLOGICAL ACTIVITIES

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Recent researches on Amaryllidaceae plant family report the isolation of more than 500 different alkaloids (AA) with different structure types possessing wide range of biological activities. Among all biological activities they have been investigated for their promising anti-tumor activity and have called attention for the ability to overcome apoptosis resistance. Among all AA, Montanine type alkaloids were intensively studied for the cytotoxicity against different human cancer cell lines. Unfortunately montanine-type alkaloids are present in plant material only in small amount. From this reason we decided to prepare series of montanine analogues from heamanthamine, which is easily isolated from plants, via intermolecular nucleophilic attack of heamanthamine.[1] So far, we prepared four new montanine derivatives and all compounds will be tested for their biological activities, and the most potential ones will be selected for further investigations.

The study was supported by SVV 260412 project.

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Thursday 24. January 2019

Lecture hall A

Session 4 - Pharmaceutical Analysis and Bioanalytical Chemistry: *8:15 – 16:00*

APPLICATION OF MONOLITHIC COLUMNS IN CLINICAL PRACTICE

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Monolithic stationary phases and columns have rapidly become highly popular separation media for liquid chromatography. Today, various types of monoliths have been developed. They have different forms and are synthesized by different preparation processes. Their unique properties distinguish them from all other columns, especially the tolerance to high flow rates achievable using only moderate pressures and high speed. This characteristics achieved excellent separation and make the columns irreplaceable in certain areas.[1,2] Monolithic columns are used for example in the analysis of biologically active substances such as neopterin, kynurenine, tryptophan and creatinine.

Elevated levels of tryptophan, its metabolite kynurenine and neopterin have been observed in disease associated with activation of immune system. Determination of these substances in biological fluids, serum, urine, wound exudates or amniotic fluid, serves to determine the patient response to therapy and clinical status. Monoliths allows large separation capacity, better separation efficiency with low back pressure and analysis of more samples of biological material compared to columns with particles.[3,4] Using of monoliths will be presented in determination of different analytes (immune system activation markers) in various biological fluids (serum, amniotic fluid, wound liquid, exudates) used in many clinical studies.

The study was supported by Project SVV 260 412, MH CZ-DRO (UHHK, 00179906) and Ministry of Health of the Czech Republic, grant nr. NV18-03-00130, NV17-29241A and NV17-28882A. All rights reserved.

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DETERMINATION OF IMPORTANT BIOMARKERS DURING TREATMENT WITH RHEOHEMAPHERESIS IN AGE-RELATED DRY FORM OF MACULAR DEGENERATION

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Age-related macular degeneration (AMD) is the most frequent cause of severe visual lost in people older than 55 years in industrial countries. The disease has two variants, dry and wet form. The most common form is the slowly progressing dry (atrophic) form. Rare (10-20 %) rapidly progressing wet form with typical neovascular choroidal membrane is in 80-90 % the cause of blindness. Rheohemapheresis is used in the treatment of dry form of AMD with soft drusen. It is a double plasma filtration in which specific high-molecular substances (LDL cholesterol, IgM, α2 macroglobulin, fibronectin, fibrinogen, von Willebrand factor) are removed. Reduction of levels of these substances leads to an improvement of the choroidal microcirculation and reduction of accumulation of lipoproteins which are the major component of soft drusen. [1]. The potential risc of this treatment method is a decrease not only in cholesterol but also in antioxidants, such as vitamin E and other important biomolecules such as vitamin A and D. For this reason, we measured levels of vitamin E (α-tocopherol), the vitamin E/cholesterol ratio in serum and lipoproteins (VLDL, LDL, HDL). Serum vitamin A (retinol) and vitamin D were also measured. These parameters were determined before and after rheohaemapheresis [2]. The individual lipoprotein layers were obtained by ultracentrifugation. The vitamins were extracted from matrix using liquid-liquid extraction, protein precipitation and filtration. For determination of vitamin E and A in serum a reversed-phase high performance liquid chromatography method using monolithic column and diode-array detection was developed and validated [3]. For determination of vitamin D was used ultra high performance liquid chromatography coupled with mass spectrometry detection (MS/MS) [4]. First results from Ministry of Health project NV17-29241A will be presented.

The study was supported by Project SVV 260 412, Ministry of Health of the Czech Republic, grant nr. NV17-29241A and NV17-28882A.

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UHPLC-MS/MS IN AN INVESTIGATION OF NOVEL CARDIOPROTECTIVE AGENT JAS-2

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JAS-2 [4,4' (Butane-2,3-diyl)bis(piperazine-2,6-dione)] is a novel analogue of dexrazoxane (DEX) which is the only approved cardioprotective agent protecting myocardium against anthracycline-induced toxicity. Despite pilot studies indicate that JAS-2 is more effective in protection of neonatal rat cardiomyocytes from toxic effect of anthracyclines as compared with DEX, its use is limited by poor solubility. Therefore, a pro-drug with a code name – GK-667 was prepared to improve the solubility of JAS-2. A modern analytical method is required to investigate stability of GK-667, its conversion to the active form – JAS-2 as well as its further metabolism. The aim of this work was UHPLC-MS/MS analysis of samples from *in vitro* experiments aimed at investigation of the prodrug activation as well as plasma from a pilot *in vivo* study. The analyses were performed using a UHPLC system (Nexera, Shimadzu,) coupled with a triple quadrupole mass spectrometer with ESI ion source (LCMS-8030, Shimadzu). The separation was achieved on Luna Omega Polar column (100 x 3.0 mm, 2.5 μm, Phenomenex) protected with a guard column. A mixture of ammonium formate and acetonitrile in a gradient mode was used as a mobile phase. Plasma samples were treated with precipitation. Cell culture medium was simply diluted with ultra-pure water. Stability study on GK-667 (100 μM, 37°C) showed similar profile in plasma and DMEM medium. GK-667 is rapidly converted to JAS-2 which is slowly degraded to JAS-2_{met}. Stability of JAS-2 (100 μM, 37°C) in both matrixes was compared with DEX. After a pilot i.v. administration of GK-667 to rabbits (5 mg/kg, i.v. n =2), only JAS-2 was detected ($c_{max} \approx 10$ μM). GK-667 and JAS-2_{met} were bellow LLOQ. The method will be further modified to enhance sensitivity, mainly for JAS-2, to be capable for full PK study in rabbits.

The study was supported by the Charles University (projects GAUK 1550217 and SVV 260 401) and the Czech Science Foundation (project 18-08169S).

DETERMINATION OF SUDAN DYES IN CHILLI PRODUCTS BY MEKC-MS/MS USING A MS FRIENDLY SURFACTANT

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Sudan dyes are phenyl-azoic derivatives widely used as synthetic organic colorants because of their colour fastness and low price. Azo dyes have been extensively used in many industrial applications including oils, solvents, plastics, etc. Sudan I, II, III, and IV have been employed for many years as food colorants in different products, such as chilli powder and sauces, to mimic, intensify, and prolong the appearance of natural red hues because of their intense red–orange colour. The use of these colorants can constitute a serious health risk, thus they are banned for food usage in the European Union since 2004[1]. The aim of this work is to develop a fast and sensitive method for the simultaneous determination of Sudan dyes (I, II, III, and IV) in chilli products such as powder, sauce and paste by micellar electrokinectic chromatography-mass spectrometry (MEKC-MS) employing ammonium perfluorooctanoate as volatile surfactant. MEKC separation and MS detection conditions have been optimized in order to achieve a fast, efficient, and sensitive separation of the four dyes. Target compounds were extracted from chilli samples with acetonitrile and purified using freezing-lipid filtration, achieving excellent results in terms of sample throughput. Analytical performance of the method is satisfactory, obtaining limits of quantification lower than 5 μg kg^{-1} in all cases. The precision, expressed as relative standard deviation (%, RSD) was below 15.7%. The extraction efficiency for fortified samples ranged from 86.5 to 99.8%, with RSDs lower than 10.3%. Matrix effect was evaluated for all samples studied, being lower than |17|% in all cases. Its applicability has been successfully tested in 20 chilli products. The concentration was calculated from the corresponding matrix-matched calibration curve, detecting Sudan I and IV in several samples at 50 and 450 μg kg^{-1} respectively.

The study was supported by the STARSS project (Reg. No. CZ.02.1.01/0.0/0.0/15_003/0000465) co-funded by ERDF.

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PROTEOMIC ANALYSIS OF ACETYLOME

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In terms of numbers, acetylation of lysine ε-amino group (AcK) represents the second most important protein posttranslational modification involved in cell signaling next to phosphorylation.[1] Unfortunately, functional role of the majority of acetylated sites (i.e. acetylome) is unknown. The position of protein acetylation remains enigmatic even for immune cells whose signaling cascades must be tightly regulated to prevent immunopathology. In particular, there are no rigorous data regarding acetylome of dendritic cells (DCs) – the most effective antigen-presenting cells responsible for priming of adaptive immunity. We therefore decided to analyze acetylation events in primary murine bone marrow-derived DCs (BMDCs) using proteomics. First, we compared AcK sites from BMDCs to those obtained from murine liver tissue. While we were able to identify >3,500 AcK sites in liver, only ~1,900 were found in BMDCs. This was probably an issue of sensitivity as AcK sites from some BMDC compartments (e.g. mitochondria) were underrepresented, suggesting low AcK stoichiometry. To further improve the depth of BMDC acetylome, we performed HPLC pre-fractionation of BMDC peptides prior immunoprecipitation step, which helped to increase the number of identified BMDC AcK sites to ~3,000. As expected from transcriptionally active immune cells, about 3% of detected acetylated BMDC proteins were transcription factors and >10% of all AcK sites were reported substrates of nuclear CBP/p300 acetyltransferase.

The study was supported by MH CZ – DRO (UHHK, 00179906).

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CHAOTROPIC AGENTS EFFECTING SEPARATION OF CHIRAL DRUG CANDIDATE K1277

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Chirality of drugs is one of major concerns since the half of the last century, when the toxicity of one enantiomer of thalidomide caused deformations in millions of new-born children in many developed countries. As a model drug for enantiomeric separation we used derivate of tacrine conjugated with tryptophan labelled K1277, which was synthetized by our colleagues as a potential antialzheimeric drug. The chromatographic separation was performed with Dionex 3000RS UHPLC system with UV detection (254 nm) in reverse phase isocratic mode with chiral Lux Cellulose-1 column as a stationary phase and a mobile phase consisting of acetonitrile and water in 45:55 or 40:60 ratio with an addition of various inorganic chaotropic agents in different concentrations. All used agents were sodium salts to minimize interactions of cationic part to the separation. The results we obtained were partly in conclusion with Hofmeister chaotropic series as reviewed by Phechkrajang[1] and used by Kazakevich *et all.*[2] and Pan *et all*[3] in their research of chaotropic behaviour. However with more potent agents the separation was still inconclusive even in the low concentrations. The research was also focused on pH addition to the effects especially in the potent agents as for example BF_4^- anion, which seemed interesting. In the future we would like to analyse behaviour of K1277 with different conditions influencing enantiomeric separation as well as using computer prediction for optimal conditions and potentially experiment with common chiral drugs.

The study was supported by Czech Science Foundation (Grant No. GA16-08554S)

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ANALYSIS OF CANNABINOIDS IN DIETARY SUPPLEMENTS AND COSMETIC PRODUCTS USING SUPERCRITICAL FLUID CHROMATOGRAPHY

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We describe development of an ultra-high-performance supercritical fluid chromatography (UHPSFC) method with PDA and MS detection for analysis of cannabinoids in dietary supplements and cosmetics products. Considering a high number of cosmetics and dietary products available on the market and process of cannabinoid legalization for medical use importance of methods suitable for quality control is growing. The optimization of UHPSFC method was carried out with mixture of six most abundant cannabinoids as reference standards including cannabidiol, Δ-9 tetrahydrocannabinol, cannabigerol, cannabidiolic acid, Δ-9 tetrahydrocannabinolic acid and cannabigerolic acid. Firstly, diol, 2-picolylamine, diethylamine, 1-aminoanthracene, BEH, BEH 2-ethylpyridine, CSH pentafluorophenyl and HSS C18 SB stationary phases were tested. Secondly, mobile phase modifiers and additives, including methanol, ethanol, mixture of acetonitrile and methanol, 0.1% ammonium hydroxide and 10 mM ammonium formate were employed. Thus, their effects on peak shape, peak resolution, selectivity, retention time and analysis time were evaluated. Other optimized parameters involved gradient elution, column temperature and pressure of back-pressure regulator. Finally, Viridis BEH 2-EP column was selected as optimal stationary phase. In order to separate the mixture of six compounds a gradient elution from 2 to 30% of solvent B was used. Mobile phase consisted of CO_2 as solvent A and methanol with 0.1% ammonium hydroxide and 2% water as solvent B. To show the applicability of the proposed method, the determination of the six cannabinoids in various cosmetics products and dietary supplements based on cannabis sativa were carried out.

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ULTRA-HIGH PERFORMANCE SUPERCRITICAL FLUID CHROMATOGRAPHY IN PHARMACEUTICAL QUALITY CONTROL: TACKLING THE METHOD VALIDATION

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First part of the study was focused on developing general achiral screening approach for ultra-high performance supercritical fluid chromatography (UHPSFC) methods. For that, 10 pharmaceutical quality control (QC) mixtures containing active pharmaceutical ingredients (API) and their particular impurities were used. In the end, not only that the screening approach was suggested based on obtained results but also UHPSFC method for each QC mixture was obtained. Even though most of these methods seemed to be sufficient, several of them had to be further optimized to ensure their successful validation and application on API and tablet samples. Several challenges occurred during the optimizations. The necessity of the resolution of API and following impurity equal at least to 3 was the most common one which was especially difficult to solve in mixtures with structurally close compounds. However, also unrepeatable elution of compound eluting close to the dead volume or in the end of gradient set-up and/or shifts of retention times due to column aging were detected. The most frequent optimization adjustments involved changes in gradient program. Moreover, the substitution of Viridis HSS C18 SB for slightly different Acquity UPLC HSS C18 SB and/or addition of acetonitrile into the modifier solution also led to significant changes in selectivity. In case of β-estradiol mixture, the coupling of columns was necessary to ensure sufficient resolution of structurally close compounds. Finally, validation of optimized methods was carried out. Parameters recommended by ICH guidelines Q2 and Q3 including specificity, linearity, range, lower and upper limit of quantification, limit of detection, accuracy, and precision were examined. Intermediate precision and the accuracy profile evaluation were also determined for several methods. In the end, all ten UHPSFC methods were successfully validated according ICH guidelines. Overall, from 55 analytes, only two impurities did not meet the validation criteria due to low sensitivity and low resolution, respectively.

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TARGETED AND NONTARGETED ANALYSIS OF HUMAN URINE FOR METABOLOMIC STUDY

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Swedish Cardio Pulmonary Bioimage Study (SCAPIS) is a project following 30 000 human subjects in the age of 50-64 years. The project was designed to improve risk prediction of cardiopulmonary disease. The Swedish SciLifeLab SCAPIS Wellness Profiling (S3WP) program consists of a smaller cohort with 100 individuals from this large SCAPIS population. It combines the use of imaging technologies, large scale omics, and epidemiological analyses. At four opportunities over one year, plasma and urine have been sampled from each subject. Urine metabolomics using ultra high-performance liquid chromatography-ion mobility-quadrupole with time of flight detector (UHPLC-IM-QTOF) is the goal of present project. A LC-MS based metabolomics method commonly used at Swedish Metabolomic Center was applied. The separation was carried out using gradient elution of mobile phase A: 0.1% formic acid, and B: 75% acetonitrile and 25% isopropanol with 0.1% of formic acid in 11.8 minutes using Acquity HSS T3 column (2.1 x 50 mm; 1.8 μm). Firstly, a retention time mass spectra library based on the Sigma Aldrich metabolite compound library, which contains 635 metabolites, was built. The retention time, collisional cross section, and mass information database were acquired for annotation of metabolites in the metabolomics analysis of the urine samples. Secondly, the dilute and shoot approach was used for sample preparation of urine samples after the optimization of dilution ratio. Based on comparison with number of detected metabolites and matrix effects, the human urine samples were diluted with 50% methanol with internal standards in a ratio 1:10. Finally, the analysis of biological samples was carried out on a sub-set of 50 individuals with the aim to detect metabolite variation within and between subjects. By using a targeted metabolomics data processing approach, in total 48 metabolites were annotated in both ionization modes. The nontargeted analysis showed 825 features in positive mode and 724 features in negative mode. Currently, the data are evaluated in the context of the whole project.

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DEVELOPMENT OF HILIC UHPLC-UV METHOD FOR ANALYSIS OF PHENOLIC COMPOUNDS

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Hydrophilic interaction chromatography (HILIC) is suitable for separation of the small polar and ionizable compounds, which are poorly retained in classical reversed phase columns. The separation mechanism is more complex than in reversed phase. It is based on combined interactions including partitioning, ion-exchange, electrostatic interactions etc. The advantages of HILIC involve a better mass spectrometry response due to high percentage of acetonitrile in mobile phase, lower viscosity, and orthogonal elution order in comparison with reversed phase. Quercetin and rutin are well known phenolic compound with many health benefits. They are metabolized to smaller phenolic acids and larger flavonoid structures. The separation of these analytes is challenging due to the differences in lipophicity, acidity, and different size of molecule. The aim of this study is to find HILIC separation conditions for analysis of 5 phenolic compounds (phloroglucinol, 3,4 dihydroxyphenylacetic acid, homovanilic acid, 3 hydroxyphenylacetic acid, 3-(3-hydroxyphenyl) propionic acid, 4-methylcatechol)) and 4 flavonoids (rutin, quercetin, tamarixetin, isorhamnetin) at MS compatible conditions. The separation potential of five HILIC stationary phases was tested, under different mobile phase composition (80-97% of acetonitrile), and different mobile phase additives including 0.1% formic acid, 10mM ammonium acetate pH 6.0, and 10mM ammonium formate pH 3.0. The most problematic molecules in terms of HILIC seem to be flavonoid molecules, where the wide peaks were observed. Tamarixetin and isorhamnetin were not separated from each other due to similar chemical properties, and showed also coelution with other compounds. The developed method is perfectly suitable for the analysis of phenolic acids. For the flavonoids molecules the other stationary phases or separation modes should be further investigated.

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DOPAMINE UNIVERSAL COATING – A NEW POTENTIAL MODIFICATION OF NANOFIBROUS SORBENTS FOR ON-LINE EXTRACTION SYSTEMS

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The dopamine universal coating showed a great potential in surface modification. 3D-structured polycaprolacton, prepared via hybrid technology combining electrospinning and meltblown, was used as a basic nanofibrous polymer for modification via polydopamine. Four groups of biologically active substances including the betablockers, bisphenols, phenolic acids and nonsteroidal drugs were tested as model analytes for the best description of the extraction behavior caused by polydopamine layer. Coated and uncoated nanofibers were successfully applied as sorbents for the on-line extraction liquid chromatography set-up and compared.
Both materials showed good potential for the determination of bisphenols and nonsteroidal drugs in samples. Polydopamine layer significantly increased the extraction efficiency for more polar drugs. All developed on-line SPE HPLC methods, were successfully applied on real samples (river water, human urine and plasma).

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APPLICATION OF NANOFIBROUS POLYMERS FOR A BIOLOGICAL SAMPLE EXTRACTION

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The sample adjustment is often discussed topic in the field of analytical separation methods. This part of analytical process is often time-consuming and laborious and it cannot be omitted. The correct sample adjustment is important step to obtain precise and accurate results. The biological samples represent specific problematics. They contain many ballast substances interfering with analytes or causing damage of instrument. In combination with low concentrations of analytes, the biological sample adjustment is necessary for sample purification and analyte preconcentration. The methods for biological sample adjustment should be fast and easy because the handling with biological fluids and tissues may be biohazardous. Therefore the automatic methods are preferred. The use of restricted access materials (RAM) meets the previous mentioned requirements. Their unique properties allow the macromolecules exclusion and analytes extraction in one step. The connection of RAM with column-switching chromatography system leads to the fully automated analytical process. First, the analytes are extracted from matrix and retained on restricted access material sorbent. Then the analytes of interest are eluted onto the analytical column and determined.

The original RAM are based on carbon or silica, the molecularly imprinted polymers, nanotubes or magnetic particles are used for modern types of sorbents. Despite their promising properties, the nanofibers did not be used. Though, our first study confirmed the statement the nanofibers are possible alternative to RAM. Nanofibrous polymers provided good extraction efficiency for parabens in human serum and bovine milk. Simultaneously, they were able to remove proteins from these matrices. Based on this obtained knowledge, the methods for determination of pharmaceutically significant substances in biological matrices are developed.

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TESTING OF NANOFIBROUS POLYMERS FOR EXTRACTION ON A MAGNETIC STIRRER

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This study was focused on nanofiber polymers and their potential use for the sorption extraction on a magnetic stirrer (SBSE - stir bar sorptive extraction). Aim of the work was to test available types of nanofibers and to prove their suitability as a material for extraction on the magnetic stirrer. The presented work included the preparation of the solutions to which we placed the magnetic stirrers coated by nanofibers where the sorptionextraction was carried out with the selected substances - Sudan dyes, NSAIDs, chlorophenols, carbamate and pyrethroid insecticides, nitrophenols, parabens and bisphenols. The chromatographic separation methods were developed for each compound of drugs separately. The substances pre-concentrated on the polymeric fibers were evaluated after chromatographic analysis and separation.

After several measurements we found out that the extreme lipophilic analytes such as Sudan dyes were irreversibly adsorbed on the polymeric fibers. The polystyrene fiber (PS) was not stable in the used organic solvents and therefore it had to be preparedagain before every extraction. In conclusion, selected kinds of the polymeric fibers which are suitable under the conditions of extraction were defined. Insecticides have been successfully pre-concentrated on polyamide 6 (PA6) with maximum obtained enrichment factor 69.4. Bisphenols have been successfully pre-concentrated on PA6 and also for composite polymer PS+ polycaprolacton (PCL) was the highest enrichment factor 82.3. The highest theoretical enrichment factor (100) has not been achieved at any of the used nanofiber.

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DESIGN OF EXPERIMENT IN SAMPLE PREPARATION FOR VITAMIN E

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Vitamin E including α-, β-, γ-, and δ-tocopherols and tocotrienols belongs among the fat-soluble compounds with important biological activity including cardioprotective, immunomodulatory, and blood cholesterol lowering effect essential for human health.. Presence of vitamin E in urine could be observed when the glomerular filtration is damaged. Due to the high lipophilicity and protein binding, liquid-liquid extraction (LLE) and protein precipitation (PP) are suitable sample preparation methods for the isolation of vitamin E from plasma and urine. The recovery of sample preparation is affected by several variables (conditions) which can be optimized using time-consuming step-by-step procedure commonly applied in many laboratories or using design of experiment (DoE). DoE involves changing all variables affecting the method recovery, identifying their interactions and relationships. It enables to determine their effect to recovery using the minimum number of experiments including different variables at different levels. The optimal conditions are achieved after the creation and evaluation of the obtained model. In this study, univariate step-by-step optimization and optimization using DoE were carried out to optimize PP and LLE. PP agent type, solvent/agent ratio, time of incubation, and time of centrifugation were tested for PP, and the type of extraction solvent, its amount, extraction time, extraction temperature, and intensity of agitation were tested for LLE to find the best conditions in the term of recovery.

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COMPARISON OF SAMPLE CLEAN-UP EFFICIENCY USING MOLECULARLY IMPRINTED POLYMER AND REVERSED PHASE SORBENT FOR ON-LINE EXTRACTION OF ZEARALENONE

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Zearalenone (ZEA) is a mycotoxin produced by *Fusarium* fungi. Despite its nonsteroidal structure, ZEA expresses estrogen-like activity and acts as endocrine disruptor in mammals. Therefore, ZEA contamination in cereal products is regulated by European legislation (EC 1126/2007).[1] Developing modern analytical methods, automation, less handling with toxic sample and significant time reducing are favorable in routine food analysis. Presented newly developed HPLC methods allowed fully automated analysis including extraction step by column switching. On-line extraction was carried out either on guard column Ascentis Express C18 or on ZEA-selective molecularly imprinted polymer (MIP). The on-line coupling was optimized in terms of solvent choice, compatibility to chromatographic separation, time programming, gradient elution, and injection volume. Following chromatographic separation was designed the same for both on-line extraction methods for their further comparison.

On-line extractions on MIP have been published scarcely due to challenging optimization and compatibility problems. On the other hand, MIP properties such as stability, robustness, and extraction capacity make it suitable for application in column-switching system. The aim of the presented study was on-line MIP SPE-HPLC method development, and its evaluation of clean-up efficiency and selectivity compared to the analogue method using conventional C18 sorbent. However, no expected significant difference in selectivity was observed. Finally, the validated methods were applied for ZEA determination in beer samples.

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STUDY OF THE BIOACCESSIBILITY OF PHTHALATES AND BISPHENOL A FROM MICROPLASTICS IN SEAWATER USING AN ON-LINE SWITCHING VALVE HPLC SYSTEM

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For the first time, an automatic flow system hyphenated to LC is presented for dynamic extraction of microplastics (polypropylene and poly(vinyl chloride)) with incurred phthalates and Bisphenol A (CRMs). This flow setup was able to mimic leaching of the additives from plastic debris in marine environment. The microplastic particles were packed into a metal column holder, through which surrogate seawater was pumped using flow technique. The bioaccessible analytes were preconcentrated on-line using a 10 mm long C18 monolithic column, placed onto the injection valve of an HPLC system, by this, also removing the seawater matrix. After loading the Bisphenol A and phthalates containing leachate, the HPLC valve was switched to the inject position and the analytes were eluted via an optimized acetonitrile/water gradient followed by UV detection. The entire method including dynamic flow-through extraction from microplastics, on-line preconcentration, and clean-up along with the HPLC separation took 24 min. Out of the 8 phthalates in CRM, only the 4 most polar species, as well as Bisphenol A, leached significantly by the seawater. To study dynamic extraction behaviour, 40 fractions were measured for each batch of particles and the elution profiles will be shown. RSD of measurements of standards was ≤ 5% and RSD of the dynamic leaching study was ≤ 11%.

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DETECTION TECHNIQUES FOR ANALYSIS OF PHENOLIC COMPOUNDS IN APPLES

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Apples represent one of the most worldwide available natural source of phenolic compounds responsible for overall well-being associated with their antioxidant properties. Selection of cultivars with higher content of phenolic compounds can improve their intake by consumption. Fruit matrices complexity and structural similarity of polyphenols increase demands on detection selectivity and sensitivity. This problem can be solved by combination of different detection principles. For this reason, fast screening HPLC method coupled with tandem detection DAD-CAD and DAD-FLD was developed to evaluate phenolic profiles in twenty apple cultivars.
Both methods, employed a fully porous particle column Luna Omega Polar C18 with stationary phase modified for separation of polar compounds. The use of linear gradient elution allowed rapid chromatographic separation. Benefits, disadvantages, and possibilities of tandem connection HPLC-DAD-CAD and HPLC-DAD-FLD methods for determination of phenolic compounds in fruit extract were summarized. DAD at 280 nm was used for determination of gallic acid, epicatechin, phloridzin, and phloretin; at 254 nm for detection of rutin and quercetin; at 320 nm detection of chlorogenic acid. CAD was universal for all analytes and FLD was used at λ_{em} 363 nm and λ_{ex} 278 nm for detection of gallic acid and epicatechin. The LOD and LOQ values of all detectors were compared. In contrast to DAD, FLD did not met the expectations for sensitive evaluation of analytes and selectivity of CAD was low due to absence of additional spectral data.

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INCORPORATION OF NEUTROPHIL ELASTASE INHIBITOR INTO POLYSULFONE MEMBRANE - NEW APPROACHES TO IMPROVE HEMODIALYSIS PATIENTS OUTCOMES

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Chronic kidney disease patients undergoing haemodialysis (HD) suffer from chronic inflammation, caused by the disease *per sé* and by the long-term contact with artificial material of HD membrane. As consequence of inflammation, the patients have elevated risk of cardiovascular diseases, and therefore markedly higher mortality rate, compared to healthy population.

One of the promising approach to diminish inflammation is to inhibit neutrophil elastase, which is excessively released from neutrophils during the HD treatment. [1] The elastase inhibition is mediated by the direct contact with elastase inhibitor incorporated into HD membrane. For this purpose, a neutrophil elastase inhibitor with chemical formula $C_{21}H_{20}N_2O_3S$ (K_i = 0.34 nM) was newly synthetized and incorporated into flat sheet polysulfone membrane during its fabrication process. The ability of modified membrane to diminish elastase activity was evaluated *in vitro,* using fluorometric assay. Membrane with identical composition, without inhibitor, was used as a blank sample.

The preliminary results show promising inhibition of elastase in physiological concentration of healthy population (10 μg L^{-1}). However, the concentration of incorporated inhibitor has to be further optimized in order to reach diminution of elastase in real values found by HD patients (29.6 – 64.8 μg L^{-1}).[2]

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COLUMN COUPLING IN SUPERCRITICAL FLUID CHROMATOGRAPHY

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Isomers and stereoisomers are always challenging to separate in pharmaceutical research because they are compounds with similar chemical properties, necessitating a unique selectivity. Coupling columns seems to be a promising approach to solve these selectivity problems. Supercritical Fluid Chromatography (SFC) has been established as a preferred chromatographic technique for chiral separations, and an increased interest in this technique is seen for achiral analyses in recent years. Supercritical fluids possess a lower viscosity compared to liquid mobile phases, which causes a lower pressure drop across the column and easily allows the coupling of several columns in series. However, retention mechanisms in SFC are more complex than in HPLC. The aim of this project is to propose a methodology to predict the retention behaviour of analytes in a coupled column system in SFC. Atenolol, ephedrine, propranolol, mianserin, labetalol, nadolol were used as chiral model analytes, quinine, quinidine as diastereomers, and aminophenol, aminobenzoic acid, aminocresol as examples of structural isomers. A combination of a chiral and an achiral stationary phase was selected for the column coupling, using Lux Cellulose-1, Lux Cellulose-2, Lux Cellulose-3, Lux Cellulose-4, Lux Amylose-2 as chiral phases and Luna NH_2, Luna Silica, Synergi Polar RP and FluoroSep-RP Phenyl as achiral phases. The mobile phase was composed of CO_2 mixed with 20 % (v/v) MeOH, which contained 0.1% (v/v) trifluoroacetic acid and 0.1% (v/v) isopropylamine. Retention factors of the coupled systems were estimated using a prediction formula.[1] That takes into account retention factors from individual stationary phases and the effective length of stationary phases. The calculated values of retention factors were compared with the experimental. Using the formula, we were able to predict elution order of the analytes in the coupled column system. Future work will focus on increasing the predicting precision of the formula by incorporating other factors that influencing the retention behaviour of analytes.

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THE DEVELOPMENT OF A LIQUID CHROMATOGRAPHY-QUADRUPOLE-TIME-OF-FLIGHT MASS SPECTROMETRY METHOD FOR DETERMINATION OF FLAVONOIDS, ISOFLAVONOID AND THEIR METABOLITES

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Known metabolites of flavonoids and isoflavonoids can be categorized into two major groups: compounds with preserved (iso)flavonoid core and low molecular weight phenolics. The aim of our project is to detect the metabolites of flavonol rutin and isoflavonoid tectoridin in rat plasma samples. Firstly, a suitable method employing ultra-high performance liquid chromatography with quadrupole-time-of-flight mass spectrometry (Q-TOF) for the analysis of tectoridin, rutin and row of its known metabolites: quercetin, quercetion-3-O-glucuronide, tamarixetin, isorhamnetin, tectorigenin, phloroglucinol, 4-methylkatechol, 3,4-dihydroxyphenylacetic acid, 3-hydroxyphenylacetic acid, homovanilic acid, 3-(3-hydroxyphenyl)propionic acid was developed. A very complex optimization of MS parameters had to be carried out especially due to the low sensitivity for the low molecular weight phenolics. The measurement was carried out in MS scan. The particular setting of Q-TOF analyzer was essential for sufficient sensitivity of phenolics, which was increased 10 times on standard solution. Secondly, the optimization of suitable sample preparation step will be necessary for the application on plasma samples. Based on the purpose of this method the simple and non-selective protein precipitation will be employed. In following step, the effect of specific Q-TOF analyzer setting on the signal to noise will be verified on matrix samples. The final method will be applied to real biological samples of rat plasma for the determination of these metabolites and for the identification of other, potentially unknown, metabolites.

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BENEFITS AND RISKS OF SEPARATIONS AT ELEVATED TEMPERATURE IN PROTEOMIC ANALYSES USING A CONVENTIONAL-FLOW LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY SYSTEM

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Since nanoelectrospray was introduced, nanoflow chromatography hyphenated via a nanoelectrospray to a mass spectrometer has generally been viewed as a *de-facto* standard platform for proteomic applications. Indeed, leveraging nanocolumns with inner diameter of 0.075 mm instead of using conventional-flow columns with inner diameter of 2.1 mm conveys a theoretical 784-fold gain in sensitivity. Nevertheless, in our recent work we demonstrated that providing the instrumentation and method are adjusted, the amount of sample for a proteomic analysis can be only roughly 5-fold greater when using conventional-flow system.[1] Column temperature is one of the parameter that contributed significantly to increasing performance of the conventional-flow system. We noticed, however, that at some point, the benefit of elevated temperature to peak shape was redeemed by lower number of identified peptides. We hypothesized that an on-column peptide degradation might occur when peptides were separated at elevated temperature using acidic mobile phases. To this end, we scrutinized the effect of temperature on the stability of a model protein trapped in a reversed phase column. We confirmed that temperature as high as 45 °C in combination with 0.1% formic acid already may induce on-column peptide bonds cleavage. We subsequently carried out data-dependent LC-MS analyses of tryptic peptides at various column temperatures. We found out that besides on-column peptide bonds cleavage, peptides trapped on a stationary reversed phase may undergo various artificial chemical modifications in the presence of 0.1% formic acid in mobile phases.

The study was supported by the STARSS project (Reg. No. CZ.02.1.01/0.0/0.0/15_003/0000465), co-funded by ERDF.

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DETERMINATION OF PHLORIDZIN AND OTHER PHENOLIC COMPOUNDS IN APPLE LEAVES, BARK AND BUDS BY HPLC

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The aim of this study was to develop a new liquid chromatography method to determine the content of phenolic compounds in raw material of apple trees – leaves, bark and buds. Raw materials from different apple cultivars were extracted in methanol acidified by 0.1% formic acid in ultrasound bath. Extracted phenolic compounds - phloridzin, phloretin, chlorogenic acid, rutin and quercitrin were subsequently analyzed by high performance liquid chromatography using YMC-Triart C18 ExRS 150 x 4.6 mm x 5um, 8 nm analytical column. The separation was performed with gradient elution at flow rate 1 ml/min. The mobile phase consisted of acetonitrile and 0.1% phosphoric acid. The detection was performed by DAD at wavelengths 280 nm, 327 nm and 354 nm. The temperature of column space was 30 °C, injection volume was 1 µl. The identification of analytes was achieved by comparing their retention time and spectra with retention time and spectra of standard solutions. The method was validated before quantification of phenolic compounds in the leaves extract. The followed validation parameters in defined ranges were obtained for the tested analytes: the linearity (R^2 = 0.994-0.998), repeatability (RSD = 0.34-1.75 %), recovery (86.54-123.21 %) and precision (RSD = 2.07-4.56 %). Phloridzin was found as a dominating phenolic compound in concentrations ranging from 42.74 to 94.96 mg/g in leaves extracts. It covered more than 90 % of total phenolic content, followed by quercitrin, chlorogenic acid, phloretin, rutin. Concentration range of phloridzin in bark extract was from 48.45 to 95.88 mg/g and in buds extract from 81.39 to 212.24 mg/g. In conclusion, the present study shows that plant material from apple trees is promising source of phytochemicals with positive effect on human health and could be used potentially for the development of dietary supplements.

Acknowledgement to financial support of Charles University SVV 260 412 and project No. QJ1510354 Creation and selection of apple varieties with high health benefits and prolonged fruit storage.

CITRININ-SELECTIVE MOLECULARLY IMPRINTED POLYMER AND ITS USE FOR ON-LINE SOLID PHASE EXTRACTION COUPLED TO LIQUID CHROMATOGRAPHY

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New selective molecularly imprinted polymer has been used for extraction in on-line SPE-HPLC to achieve the selective determination of citrinin. Four different imprinted polymers varying in combinations of components prepared by bulk polymerization were evaluated in terms of binding capacity and selectivity. Imprinted polymer prepared from a mixture comprising 1-hydroxy-2-naphtoic acid as the template molecule, acrylamide as the structural monomer, ethylene dimethacrylate as the crosslinker (in a molar ratio of 1:4:16), and acetonitrile as the porogenic solvent exhibited the best properties. Selectivity of this sorbent was confirmed by comparison with the non-imprinted counterpart prepared using the same polymerization carried out in absence of the template. Imprinted polymer was packed in a 20 × 3 mm i.d. steel cartridge and coupled to the on-line SPE-HPLC system through a six-port switching valve. The method for determination of citrinin including the on-line extraction step was then developed and validated. The sample in the form of methanolic extract was loaded, cleaned, and preconcentrated in the imprinted SPE cartridge. Following separation of citrinin from residual interferences was achieved using analytical column Kinetex Biphenyl 100 × 4.6 mm i.d., 5 μm particle size, and fluorescence detection (Ex 335, Em 500 nm). The total analysis time was only 9.50 min. Fully validated method was also applied to analysis of food supplements based on red yeast rice extracts which control is implemented in European legislation. Only minor yet acceptable contamination was found in tested samples.

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COMPARISON OF LIPID EXTRACTION METHODS FROM MILK USING 2D LIQUID CHROMATOGRAPHY – MASS SPECTROMETRY

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Over the past decades, lipid samples were mainly extracted using labor intensive, non-parallelizable methods such as Bligh & Dyer and Folch extraction using toxic non-polar solvents e.g. chloroform. However, these methods were developed when high-resolution accurate mass measurements instruments were not widely available. In addition, medical interest in short and medium chained triacylglycerols was disclosed recently. Comparison of these methods with more modern methods such as single extraction solvent mixture and methyl *tert*-butyl ether extraction was carried out. Major benefit of these methods compared to traditional extraction is wider coverage of extracted lipids.

Chromatographic system used was two-dimensional liquid chromatography using non-aqueous reverse phase on C18 stationary phase in the first dimension and silver-ion stationary phase in the second dimension. Mass spectra were acquired using parallel outlet to high-resolution accurate mass measurement spectrometer with electrospray ionization and triple-quadrupole with atmospheric pressure chemical ionization in first dimension. Second dimension contained parallel outlet to the two triple-quadrupole with to atmospheric pressure photoionization and ion trap with electrospray ionization.

The study was supported by the STARSS project Reg.No.CZ.02.1.01/0.0/0.0/15_003/0000465) co-funded by ERDF and SVV 260 412/2018.

Thursday 24. January 2019

Lecture hall B

Session 5 - Pathobiochemistry and Xenobiochemistry: 8:15 – 11:15

ANTHELMINTICS IN PLANTS

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Anthelmintics, the drugs against parasitic worms, are widely used in human and veterinary medicine, nowadays. The usefulness of anthelmintic drugs is indisputable, but at the same time they pose a risk to ecosystems. With excrements of treated animals, anthelmintics can get into the environment and there affect non-target organisms – free-living invertebrates and wild plants. In our project, we are focused on antiparasitical drugs ant theit uptake, biotransformation and transcriptional response in plants. The most frequently used anthelmintics (albendazole, fenbendazole, flubendazole, ivermectin, monepantel) are used, and different plant species are tested, also the model plant *Arabidopsis thaliana* (wild type, *Brassicaceae*).

The presented work is the part of this project. The aim of the study is to get information about the effects of anthelmintics on hydroponics cultures of *Arabidopsis thaliana* presented as changes in plant transcriptome. The broad-spectrum benzimidazole anthelmintic fenbendazole and macrocyclic lactone ivermectin were used. Hydroponics cultures was stressed by 5 μM anthelmintic. The effect was studied after 24 and 72 hours of stress. The microarray analysis was performed. For general expression at the transcription level were used Agilent-based microarrays. Quantitative analysis of whole proteomes comparing fenbendazole treated and control samples was carried out using a LC/MS Thermo Orbitrap Fusion spectrometer.

In our study the presence of fenbendazole or ivermectin and theirs metabolites in *Arabidopsis thaliana* influenced gene expression. It was more affected by both anthelmintic in rosettes than in roots. More than 4.000 proteins were identified by proteomic analysis. Most proteins were identified in leaves of plants stressed by fenbendazole.

The study was supported by by the Czech Science Foundation (GA ČR, grant No. 18-08452S).

SELECTION AND VALIDATION OF REFERENCE GENES FOR mRNA AND miRNA GENE EXPRESSION STUDIES IN HUMAN LIVER SLICES USING RT-qPCR

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Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) is a frequently used technique for gene expression profiling. RT-qPCR analysis depends on data normalization by endogenous reference gene, which expression is stable and independent on experimental conditions. Precision-cut liver slices (PCLS) are an interesting model due to its multicellular composition, preserved tissue architecture and intercellular communication. Its applicability to human tissues allows us to avoid interspecies differences and directly apply human tissues into multiple experimental designs. PCLS also represent a promising model for gene expression studies. However, there exists no study validating this model for selection of a suitable reference gene (or their combination). Therefore, we decided to perform a validation study, since selection of inappropriate reference gene can influence the trend and deviation of results. Three human liver samples received from surgery were used to obtain PCLS (8 mm diameter, 150 μm thickness), which were cultivated for 24 hours and samples were collected after 0, 4, 8, 12, 18 and 24 hours. As we are interested in induction studies, two cytochrome P450 (CYP) inducers β-naphthoflavone and rifampicine were used as positive controls. To verify the viability of PCLS, ATP content and lactate dehydrogenase leakage were measured. Based on literature review, six candidates (*GAPDH*, *SDHA*, *ACTB*, *B2M*, *HPRT* and *YHWAZ*) and five candidates (miR-16-5p, miR-23b-3p, miR-93-5p, miR152-3p and U6) were selected for mRNA and miRNA validation, respectively. Stability of those genes was compared using RefFinder, a free web tool that uses several other software, such as geNorm, Normfinder, BestKeeper and the comparative Ct method. Stability of selected reference genes for mRNA was validated on expression analysis of *CYP3A4* and *CYP1A2*.

The study was supported by the Czech Science Foundation (grant No. 18-09946S)

PHENOTYPIC SCREENING OF A CHEMICALLY DIVERSE COMPOUND LIBRARY IDENTIFIED TWO COMPOUNDS WITH ANTHELMINTIC ACTIVITY AGAINST *HAEMONCHUS CONTORTUS*

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Due to the widespread development of anthelmintic resistance in *Haemonchus contortus*, there is a continuing need to discover and develop new anthelmintic drugs to ensure sustainable control of this and related economically important and pathogenic nematodes of ruminants. With this focus in mind, we screened a compound library consisting of 236 chemicals representing diverse classes such as hetorocyclic compounds (e.g. thiazoles, pyrroles, quinolines, pyrimidines, benzo[1,4]diazepines), hydoxamic acid-based metalloenzyme inhibitors, peptidomimetics (bis- and tris-pyrimidoneamides, alkoxyamides) and various intermediates. In the present study, we measured the inhibition of larval motility and development of exsheathed third-stage (xL3) and fourth-stage (L4) larvae of *H. contortus* using an optimised, whole-organism phenotypic screening assay. Of the 236 compounds, we identified two active compounds (called BLK127 and HBK4) which induced phenotypic changes in the worm *ex vivo*. Compound BLK127 induced an 'eviscerated' phenotype in xL3 larvae and also inhibited L4 larvae development. Compound HBK4 exerted a 'curved' phenotype in both xL3 and L4 larvae. The findings from this study provide a sound basis for future work on assessing the activity of the compounds identified here on adult stages of *H. contortus* both *ex vivo* and *in vivo* (within the host animal), and against other parasitic worms of veterinary and medical importance.

The study was supported by SVV260416 and EFSA-CDN No. CZ.02.1.01/0.0/0.0/16_019/0000841 and by the Strategic Partnerships Fund of Charles University.

THE ROLE OF THE UDP-GLYCOSYLTRANSFERASES IN THE METABOLISM OF ANTHELMINTICS IN *HAEMONCHUS CONTORTUS*

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Haemonchus contortus, a hematophagous gastrointestinal parasite, endanger predominantly small ruminants, such as sheep and goats, has a great ability to develop resistance to anthelmintic drugs. Therefore, it is necessary to understand the mechanism of the resistance. The effects of anthelmintics can be reduced by xenobiotic-metabolizing enzymes by decreasing the concentration of the active drugs in parasite cells. UDP-glycosyltransferases (UGTs), important enzymes in the metabolism of xenobiotics and eobiotics, could protect the helminth from toxic action of anthelmintics by their metabolism to inactive glycosides. The previous study show that albendazole, ricobendazole and flubendazole underwent glycosidations caused by UDP-glycosyltransferases. Except sex-differences in anthelmintics metabolism, more glycosylated metabolites were observed in IRE (benzimidazol resistant) strain than ISE (sensitive) strain of *H. contortus*.[1] This analysis confirmed the connection between anthelmintics resistance and their metabolism. Using quantitative PCR, the differences in the constitutive expression of UGTs between IRE and ISE strains were analyzed. Several enzymes from the UGTs superfamily, e.g. UGT368B2, were significantly more expressed in IRE strain than ISE strain.[2] The enhanced expression of biotransformation enzymes could lead to increased rate of anthelmintics metabolism.

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BUPARLISIB IS A NOVEL INHIBITOR OF DAUNORUBICIN REDUCTION MEDIATED BY ALDO KETO REDUCTASE 1C3.

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Buparlisib is a pan-class I phosphoinositide 3-kinase (PI3K) inhibitor and is currently under clinical evaluation for the treatment of different cancers. Because PI3K signalling is related to cell proliferation and resistance to chemotherapy, new therapeutic approaches are focused on combining PI3K inhibitors with other anti-cancer therapeutics. Carbonyl-reducing enzymes catalyse metabolic detoxification of anthracyclines and reduce their cytotoxicity. In the present work, the effects of buparlisib were tested on five human recombinant carbonyl-reducing enzymes: AKR1A1, AKR1B10, AKR1C3, and AKR7A2 from the aldo-keto reductase superfamily and CBR1 from the short-chain dehydrogenase/reductase superfamily, all of which participate in the metabolism of daunorubicin. Buparlisib exhibited the strongest inhibitory effect on recombinant AKR1C3, with a half-maximal inhibitory concentration (IC_{50}) of 9.5 μM. Its inhibition constant K_i was found to be 9.9 μM, and the inhibition data best fitted a non-competitive mode. The same extent of inhibition was observed at the cellular level in the human colorectal carcinoma HCT 116 cell line transfected with a plasmid encoding the *AKR1C3* transcript (IC_{50} = 7.9 μM). Furthermore, we performed an analysis of flexible docking between buparlisib and AKR1C3 and found that buparlisib probably occupies a part of the binding site for a cofactor most likely via the trifluoromethyl group of buparlisib interacting with catalytic residue Tyr55. In conclusion, our results show a novel PI3K-independent effect of buparlisib that may improve therapeutic efficacy and safety of daunorubicin by preventing its metabolism by AKR1C3.

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CYTOCHROME P450 IN *HAEMONCHUS CONTORTUS*

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Resistance to anthelmintic drugs has become a major concern worldwide in animal health. The nematode *Haemonchus contortus*, one of the most economically important parasite of small ruminants, has become multi-resistant for all classes of used and known anthelmintics. One possible mechanism of resistance development is increased expression of drug metabolizing enzymes, cytochromes P450 (CYPs). *H. contortus* genome contains around 18 CYPs homologs and some of them might to play an important role in resistance development[1,2]. The aim of this study is to specify which CYPs expression differ in drug-sensitive and drug-resistant adults of *H. contortus*. In addition, the potential inducibility of these genes by different xenobiotics exposure was tested. Three isolates of *H. contortus* adults, susceptible ISE, resistant to ivermectin (IVM) IRE and multi-resistance WR, were obtained using agar method from lambs' abomasa and sexed based on morphology. Following incubation with different xenobiotic (IVM, ABZ, MOP, PHB, BNF) the expression of CYPs was analyzed by qPCR. The constitutive expression of several CYPs differs between sex and between all three isolates. Increased expression of *cyp7*, *cyp8* was observed in WR females, while *cyp2* is overexpressed in IRE females. In comparison to males, where the overexpression of *cyp2*, *cyp7*, *cyp8* is significant in both IRE and WR isolates. Several CYPs are significantly inducible with various xenobiotics especially in susceptible isolate. Our data suggest that some CYPs might be resistance related. Identification of such CYPs would lead to important biological marker in anthelmintic development.

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17β-HSD10 INHIBITORS AS A POTENTIAL THERAPY IN NEURODEGENERATIVE DISORDERS

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Mitochondria are the unique organels of many cell processes including energetic metabolism, maintaining homeostasis, or regulation of cell death, and are an important player in many neurodegenerative disorders.

17β-hydroxysteroid dehydrogenase type 10 (17β-HSD10) is a mitochondrial protein playing important role in various physiological pathways, mainly in steroid metabolism, but was also found to be overexpressed in Alzheimer's disease (AD) and several types of cancer. In AD 17β-HSD10 can bind β-amyloid resulting in increased oxidative stress, cell toxicity, and neuronal impairment[1]. Modulation of this enzyme could be a novel target for neurodegenerative disorders treatment[2].

For testing of various novel 1-(benzo[d]thiazol-2-yl)-3-phenylurea-based inhibitors[3-5], the recombinant enzyme was produced in *E. coli* and purified using chromatographic methods. Enzymatic activity assay was performed spectrophotometrically in a microplate reader at 37 °C, using acetoacetyl-CoA as a substrate. Kinetic parameters of enzyme were determined and over 200 inhibitors were screened for their inhibitory ability. The most inhibiting compounds were selected and their IC_{50} constants and type of inhibition were determined. These compounds are further studied using *in vitro* and *in vivo* methods with implication to neurodegenerative disorders and/or cancer.

The study was supported by the Ministry of Health of the Czech Republic (No. NV15-28967A) and Specific Research Project of Faculty of Science, University of Hradec Kralove (No. 2115-2018).

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EVALUATION OF PROTECTIVE PROPERTIES OF DEXRAZOXANE AND OTHER CATALYTIC INHIBITORS OF TOPOISOMERASE II AGAINST ANTHRACYCLINE CARDIOTOXICITY *IN VITRO*

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Anthracyclines (ANT) are one of the most effective anticancer drugs, but their use is limited by their irreversible cardiotoxicity. ANT belong to topoisomerase II (TOPII) poisons, giving rise to DNA double strand breaks. Poisoning of the TOPIIα in cancer cells is one of the fundamental mechanisms of their antineoplastic action, but their effect on the other isoform, TOPIIβ, could play a crucial role in development of their cardiotoxic effects. However, the exact mechanism of their cardiotoxicity is still not completely understood, which hampers the rational approach to discovery of protective strategies. Dexrazoxane (DEX) is the only compound that has shown considerable cardioprotective potential against ANT cardiotoxicity in experimental studies as well as in randomized clinical trials. Latest studies suggest that the cardioprotective activity might be associated with its catalytic inhibition of TOPII. This hypothesis is supported by the results of our previous study, where not only DEX but also structurally different TOPII catalytic inhibitor merbarone showed protective potential in neonatal rat cardiomyocytes without compromising the daunorubicin (DAU) antiproliferative effect.

Therefore, we decided to study the cardioprotective potential of several other structurally different compounds, which have been described as catalytic inhibitors of TOPII (suramin, aclarubicin and gambogic acid) or as TOPII poisons (genistein and XK-469, the latter described to be specific for the beta isoform of this enzyme). In this study, using primary cultures of isolated rat neonatal cardiomyocytes, we studied their own toxicity and the protective effects against cardiotoxicity caused by DAU and compared the effects of these compounds to the protective effect of DEX in our previously established model, using clinically relevant concentrations of DAU. Aclarubicin, gambogic acid and genistein did not show any cytoprotective effect against toxicity caused by DAU. On the other hand, both suramin (catalytic inhibitor) and XK-469 (topoisomerase poison) both displayed significant protective potential in neonatal cardiomyocytes, although their effect was not better than efficiency of DEX.

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SYNERGISTIC EFFECT BETWEEN MIDOSTAURIN AND DAUNORUBICIN IS RELATED TO ALDO-KETO REDUCTASE 1C3 INHIBITION

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Midostaurin is a tyrosine kinase 3 (FLT3) inhibitor (TKI) acting against targets known to be expressed in hematologic disorders[1]. FDA recently approved midostaurin combined with daunorubicin chemotherapy for the treatment of acute myeloid leukemia (AML)[2]. Aldo-keto reductase 1C3 is an enzyme overexpressed in a range of cancer types and leukemia cell lines[3] which reduces daunorubicin into inactive hydroxy metabolite daunorubicinol, thus conferring resistance to this chemotherapeutic[4]. Here, we report that midostaurin is a strong inhibitor of daunorubicin reduction mediated by human recombinant AKR1C3. On the other hand, we performed cytotoxic studies with KG1a, a myelogenous leukemia cell line who express high amounts of AKR1C3. In these studies, midostaurin significantly sensitized KG1a cells to cytotoxic effect of daunorubicin. In addition, downregulation of AKR1C3 by siRNA reduced synergistic effect between midostaurin and daunorubicin in KG1a cells. In conclusion, our results demonstrate a novel effect of midostaurin that could contribute to further understanding of the effectivity of midostaurin and daunorubicin combined therapy in clinical practice.

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THE EFFECT OF SESQUITERPENES ON ENZYMES INVOLVED IN DETOXIFICATION PROCESSES IN HUMANS

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Sesquiterpenes β-caryophyllene (CAR), caryophyllene oxide (CAO), α-humulene (HUM), farnesol (FAR), *cis*-nerolidol (cNER) and *trans*-nerolidol (tNER) possess numerous biological and pharmacological activities, among which an inhibitory effect on several phase I drug-metabolizing enzymes (DME) in human and rat subcellular fractions have been observed. Since they are often present in traditional medicinal products as well as in human diet, we intended to investigate the effect of these sesquiterpenes on the mRNA and protein expression of several DME, namely cytochrome P450 (CYP) isoforms 3A4 and 2C, carbonyl reductase 1 (CBR1) and aldo-keto reductase 1C (AKR1C), in human liver. For the investigation, freshly prepared precision-cut liver slices were cultivated in a medium supplemented with studied sesquiterpenes at 10 μM concentration for 24 h. Real-time quantitative PCR method was used to determine the mRNA levels of the studied DME, the protein levels were detected using western blot technique. So far, the results suggest that FAR and tNER possess the ability to negatively affect the levels of mRNA of all studied DME. Moreover, CAR, CAO and HUM also inhibited the expression of mRNA of all DME, except for CYP2C. Their effects, however, differ among individual human liver samples presumably due to possible inter-individual variability. Such variability may also be seen when concerning the changes in protein expression. For instance, in case of FAR and tNER, both, an inhibitory as well as an inductive effect on protein expression in human liver was observed. cNER seems to have no effect on the tested DME. The influence of CAR, CAO and HUM on protein expression is yet to be studied.

This study was supported by the Czech Science Foundation, Grant No. 18-09946S and P303/12/G163.

INVESTIGATION OF DNA DAMAGE IN ANTHRACYCLINE-INDUCED CARDIOTOXICITY USING THE COMET ASSAY AND H2AX PHOSPHORYLATION

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Despite their long history, anthracycline antibiotics (ANT) are still used in clinics in various antineoplastic protocols. Their usage is hampered by several life-threatening side effects such as myelotoxicity and cardiotoxicity. The mechanism of cardiotoxicity remains obscure, limiting the rational discovery of protective strategies. Anthracyclines belong to Topoisomerase II (TOPII) poisons, giving rise to DNA double strand breaks. Poisoning the TOPIIα in cancer cells provides the basis of their antineoplastic action, but it can also play a role in cardiotoxicity. As TOPIIα expression is minimal in post-mitotic cells, TOPIIβ is the main isoform present in quiescent cells (e.g. cardiomyocytes) and has distinct functions from TOPIIα, involving gene expression regulation and DNA repair. The DNA damage generated in cardiomyocytes by ANTs could lead to the longterm effect on the heart. Moreover, ANTs could also cause oxidative DNA damage through redox-cycling mechanisms. To assess the possible role of DNA damage, several distinct approaches can be employed. Directly, the DNA double strand breaks (DSB) can be assayed by single-cell gel electrophoresis "the comet assay", where purified nucleoids of single cells trapped in agarose are subjected to electric field. Each DSB loosens the loops in the nucleoid, forming a comet-like image. Another method involves the assessment of histone H2AX which is phosphorylated consequently to the DSB formation. These two methods were used to investigate the level of DNA damage caused by ANTs in isolated rat neonatal ventricular cardiomyocytes and HL-60 cells. Furthermore, dexrazoxane as the only approved cardioprotective agent and TOPII catalytic inhibitor was used in analysis ANT-induced DSB formation.

The study was supported by Czech Science Foundation (project 18-08169S) and fund SVV 260 416.

Thursday 24. January 2019

Lecture hall B

Session 6 - Pharmacology and Toxicology: 12:30 – 17:00

INSIGHT INTO A REGULATORY NETWORK OF PREGNANE X RECEPTOR EXPRESSION

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The pregnane X receptor (PXR, NR1I2) is a key transcription factor involved in a regulation of both endogenous and exogenous metabolism. As a ligand-activated nuclear receptor, PXR responds to the structurally diverse collection of compounds. Following its activation, PXR triggers transcription of CYP3A4, pivotal Phase I metabolic enzyme which is estimated to metabolise approximately 50 % of all marketed drugs. Of particular interest, the drug-mediated activation of PXR-CYP3A4 axis may result in clinically significant pharmacokinetic interactions.

Although, PXR has been studied intensively regarding its transcription function, less is known about exact mechanisms standing behind its own regulation. As previously shown, PXR expression is under the control of activated glucocorticoid receptor (GR) which directly increases a level of PXR transcript. In present follow-up study, we demonstrate that PXR mRNA is not only induced at transcriptional level but also stabilised at post-transcriptional level after activation of GR.

There will be provided an evidence during the lecture that GR mediates changes in miRNA expression which may subsequently lead to stabilisation of PXR mRNA via its 3´-untranslated region. The given postulate will be supported by data gained from gene reporter studies using various reporter vectors, miRNA expression profiling, and qPCR.

Overall, major conclusion drawn from our work is that GR increases expression of PXR mRNA by dual mechanisms such as transcriptional activation of PXR from its promoter and induction of post-transcriptional stabilisation.

The study was supported by the project EFSA-CDN [No.CZ.02.1.01/0.0/0.0/16_019/0000841] co-funded by ERDF.

NOVEL OBETICHOLIC ACID KETODERIVATIVES AND ISOMERS AS POTENTIAL LIGANDS OF BILE ACID RECEPTORS

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Metabolic diseases with altered cholesterol and triglyceride levels are serious healthcare problem, emerging in western population, and are tightly linked to inflammation. Recently, obeticholic acid (OCA), a potent farnesoid X receptor (FXR) agonist, has been shown to be a promising treatment against inflammatory hepatic disorders. Therefore, we aimed to synthesize new derivatives and isomers of OCA (named A-I) and assess their capacity to activate nuclear receptors involved in metabolic regulation including FXR, vitamin D receptor (VDR), pregnane x receptor (PXR) and constitutive androstane receptor (CAR).[1, 2] Gene reporter assays were performed to determine their capacity to activate nuclear receptors of interest and changes on expression of target genes were analysed by real time qPCR. Furthermore, these isomers were subjected to LC/MS analysis to determine their stability and possible conversion to OCA in HepG2 cell line and primary human hepatocytes. Our results showed that all derivatives could significantly activate FXR and PXR in therapeutic doses. Compound G is an equally strong ligand of FXR as OCA itself. We have also found that compound H is an activator of VDR. None of the tested compounds was able to activate CAR. Here, we have presented novel ligands of bile acid nuclear receptors derived from OCA. Moreover, we have found a new dual FXR/VDR agonist, compound H, which may have a promising use in the therapy of inflammatory metabolic disorder including steatohepatitis or atherosclerosis.[3]

The study was supported by GAUK 170/50/85006 and SVV 260 414.

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ACTIVATION OF HUMAN CONSTITUTIVE ANDROSTANE RECEPTOR (CAR) AGONIST BY BENZODIAZEPINES WITHOUT PROLIFERATIVE EFFECT AND LIVER TUMORIGENIC EFFECTS IN HUMAN CELLS

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A large number of nongenotoxic chemicals have been shown to increase the incidence of liver tumours in mice by a mode of action involving activation of the constitutive androstane receptor (CAR). Studies with the model CAR activator phenobarbital (PB) have demonstrated that events for mouse liver tumour formation include CAR activation, increased hepatocyte replicative DNA synthesis (RDS), induction of cytochrome P450 CYP2B subfamily enzymes, liver hypertrophy, increased altered hepatic foci and hepatocellular adenomas/ carcinomas. However, this phenomenon is not confirmed in humans. In the study, we examined drugs widely used in clinics for their interaction with human CAR. We found that the some benzodiazepines significantly activate human CAR in gene reporter assay and stimulated CAR translocation in vitro. However, benzodiazepines did not stimulate expression of genes involved in CAR-mediated hepatocyte proliferation in HepaRG cells. Diazepam did not stimulate cell cycle or anti-apoptotic gene expression. We did not find indication about live tumor promoting effect in databases. We can conclude that some benzodiazepines are CAR activator that unlikely stimulate proliferation or liver tumor promotion in humans.

The study was supported by GAUK 170/50/75006.

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INTERPLAY OF ABCB1, ABCC1 AND OATPS TRANSPORTERS IN TRANSFER OF MARAVIROC ACROSS THE HUMAN PLACENTA

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Maraviroc was developed as an inhibitor of HIV entry co-receptor CCR5. Due to importance of CCR5 in physiological signalling processes in organism and also between tumour cells the drug is currently evaluated as a new drug in treatment of many inflammatory and cancer diseases including states that occur in pregnant women. We have recently shown that maraviroc is transported by human ABC drug efflux transporter ABCB1 that is considered as protective component of placental barrier. In this study we aimed to verify the role of this transporter in limiting maraviroc distribution across the human placenta. The method of closed-circuit dual perfusion of maraviroc across human placental cotyledon was performed showing slight decrease of maraviroc concentration in fetal compartment. Presence of ABCB1 inhibitor elacridar and ritonavir abolished this decline, confirming ABCB1-mediated efflux of maraviroc in the feto-maternal direction. However, accelerated transport of maraviroc in materno-fetal direction was also found, suggesting contribution of another transport mechanism in the opposite direction to ABCB1. Bi-directional transport study in monolayers of MDCKII-ABCC1 cells and accumulation study in A431-OATP2B1, -OATP1A2 and –OATP1B3 were performed, identifying maraviroc as substrate of human ABCC1, OATP1A2 and OATP1B3 . Gene expression of ABCC1 and OATP1A2 was subsequently confirmed in all the perfused placentas well as in isolated trophoblast and fetal endothelial cells. Our data thereby indicate interplay between ABCB1 acting in feto-maternal direction and other transporters acting in materno-fetal direction, most probably ABCC1 with possible contribution of OATP1A2. These findings may help to understand maraviroc pharmacokinetics in pregnant women and contribute to safer therapy. Moreover, they indicate that the protective role of ABCB1 in the placenta may be covered up by other transporters promoting the transfer in the opposite, materno-fetal direction.

The study was supported by the Grant Agency of Charles University (grant No. GAUK 616216/C/2016), the Czech Science Foundation (grant No. GACR 17-16169S) and SVV/2017/260-414.

PLACENTAL TRANSPORT AND METABOLISM OF SEROTONIN AND TRYPTOPHAN

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Serotonin (5-HT) is an important neurotransmitter critical for fetal brain development and programming during pregnancy. To date, however, controversies remain on the source of fetal 5-HT. Until recently, it was believed that maternal 5-HT crosses the placenta easily and plays a key role in maintaining fetal brain levels. Nevertheless, recent studies demonstrate that 5-HT can also be synthesized from tryptophan (TRP) within the trophoblast. In addition, kynurenine pathway of TRP metabolism has also been identified in the placenta, giving rise to neuroprotective and neurotoxic metabolites. Furthermore, expression and activity of the rate-limiting enzymes of TRP metabolism in the placenta may be perturbed by maternal conditions such as inflammation, stress, or depression, recently associated with various disorders in fetal neurodevelopment. In our project we aim to characterize and validate several *in vitro/in situ/ex vivo* placental models to investigate possible effects of pathologies (e.g. infections) and pharmacotherapy (e.g. antidepressants) on transport and metabolism of TRP and 5-HT in the feto-placental unit.

qRT-PCR was utilized to analyze the expression of 29 transporters/enzymes in first trimester and term human placentas, rat term placentas and placental derived cell lines. Furthermore, HPLC analytical methods have been developed for quantification of TRP and 5-HT in biological samples. Dually perfused rat term placenta was employed to investigate placental transport and catabolism of TRP and 5-HT. Finally, 5-HT uptake was quantified using *in vitro* (BeWo choriocarcinoma cell line) and *ex vivo* (fresh villous fragments isolated from human term placenta) models.

The study was supported by the Charles University (Progres Q42).

INTERACTIONS OF ABC TRANSPORTERS AND CYTOCHROME P450 ISOFORMS WITH THE TYROSINE KINASE INHIBITOR ENSARTINIB

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Ensartinib is a promising small molecule tyrosine kinase inhibitor which is currently evaluated in phase II/III clinical trials for the treatment of solid tumors. In our research, we focused on the interactions of ensartinib with ABC drug efflux transporters and CYP450 biotransformation enzymes, important proteins that control pharmacokinetic behavior of number of drugs and also play the role in cancer multidrug resistance (MDR). First, we assessed the inhibitory effect of ensartinib on human ABCB1, ABCG2 and ABCC1 transporters using accumulation assays in MDCKII cells overexpressing respective ABC transporters. The results of these experiments showed that ensartinib is a potent inhibitor of ABCB1 and ABCG2. Consequently, the potential of ensartinib to overcome ABC transporter-mediated cytostatic MDR was evaluated in the follow-up MTT drug combination studies. In these assays, ensartinib effectively reversed daunorubicin and mitoxantrone resistance in MDCKII cells with ABCB1 and ABCG2 overexpression, respectively. Furthermore, qRT-PCR gene expression studies were performed in A549, CaCo2, NCI-H1299, and LS174T cellular models; we recorded no significant induction of *ABCB1*, *ABCG2* or *ABCC1* genes in all the cell lines following exposure to ensartinib. Finally, using Vivid CYP450 screening kits, we demonstrated that ensartinib is a strong inhibitor of CYP3A4, CYP3A5, CYP2C9 and CYP2C19, a moderate inhibitor of CYP2C8 and CYP2D6 and does not affect the activity of CYP1A2 and CYP2B6 isoforms. In conclusion, our findings indicate that ensartinib exhibits potential to provoke drug-drug interactions and affect MDR phenotype via changes in the activity but not in gene expression of particular ABC transporters and biotransformation enzymes. Moreover, our *in vitro* combination experiments revealed possible new effective therapeutic approach targeting ABCB1 and/or ABCG2 overexpressing tumors, which could serve as a valuable foundation for future *in vivo* studies.

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INTERACTION OF PROTEIN KINASE INHIBITORS WITH ABC TRANSPORTERS IN ACUTE MYELOID LEUKEMIA, POTENTIAL ROLE IN DRUG RESISTANCE

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Acute myeloid leukemia (AML) represents a severe hematological malignity, which is, despite the overall therapeutic advances, still characterized by poor prognosis and short survival. These unfavorable characteristics are associated also with the expression of ATP-binding cassette (ABC) transporters in undifferentiated blast cells that drive disease progression and relapses. Our aim was to investigate the interactions of modern protein kinase inhibitors abemaciclib, palbociclib, ribociclib and midostaurin, the novel drugs that have recently gained a breakthrough approval in treatment of both solid tumors and leukemia, with ABC transporters. *In vitro* and *ex vivo* approaches comprising cellular models of resistant HL-60 cells and *ex vivo* peripheral blood mononuclear cells (PBMCs) isolated from *de novo* diagnosed AML patients were employed.

Accumulation studies in HL-60 cells and their ABCB1- and ABCG2-overexpressing variants confirmed that all the tested drugs show ability to inhibit efflux of daunorubicin and mitoxantrone, the anticancer drugs approved by US Food and Drug Administration for AML therapy and at the same time substrates of both, ABCB1 and ABCG2 transporters. Furthermore, the ABCG2 inhibitors effectively enhanced number of apoptotic HL-60-ABCG2 cells, when combined with mitoxantrone, as confirmed by Annexin V/propidium iodide double staining. Abemaciclib, ribociclib and midostaurin applied in human plasma-relevant concentrations further increased mitoxantrone accumulation in PBMCs of AML patients.

To conclude, our preliminary results indicate the ability of protein kinase inhibitors abemaciclib, ribociclib and midostaurine to interact with ABC transporters in AML patient-derived cells. These data will form a basis for our follow up research on AML resistance mechanisms and approaches for their possible overcoming.

The study was supported by Charles University (SVV/260-414) and by EFSA-CDN: CZ.02.1.01/0.0/0.0/16_019/0000841.

DISCOVERY OF A NOVEL MOUSE CONSTITUTIVE ANDROSTANE (CAR) RECEPTOR AGONIST THAT DOES NOT POSSESS PROLIFERATIVE ACTIVITY CONNECTED WITH NON-GENOTOXIC HEPATOCARCINOGENESIS IN MICE

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Constitutive Androstane Receptor (CAR) is the primary regulator of drug metabolism and detoxification. CAR activation is connected with mitogenic effects leading to liver hypertrophy and tumorigenesis in rodents. Recently, stilbenoids resveratrol and *trans*-3,4,5,4´-tetramethoxystilbene (TMS) have been shown to abrogate or alleviate *N*-nitrosodiethylamine/PB-induced liver carcinogenesis or oxidative stress signaling.

Thus, we examined if TMS may be an inverse agonist of mouse Car. Unexpectedly, we have identified TMS as a novel moderate murine Car agonist in cellular reporter gene experiments, in *in silico* docking experiments as well as in induction experiments in mouse hepatocytes, in AML-12 hepatic cells, or in mice. TMS significantly up-regulates *Cyp2b10*, *Cyp2c29* and *Cyp2c59* mRNAs, but down-regulates expression of genes involved in gluconeogenesis and lipogenesis such as *Pck1*, *G6pc*, *Scd1*, *Acaca* and *Fasn* in similar degree as TCPOBOP. Importantly, TMS does not induce genes involved in liver proliferation or apoptosis such as *Mki67*, *Foxm1*, *Myc*, *Mcl1*, *Pcna*, *Bcl2*, *Bax* or *Mdm2* in C57BL/6 mice, and has no statistically significant effects on Ki67 and Pcna labeling indexes in mice, but slightly up-regulates *Gadd45β* mRNA expression.

We can thus conclude that TMS is a novel mouse Car ligand with limited effects on hepatocyte proliferation, but controlling Car-target genes involved both in xenobiotic and endobiotic metabolism.

The study was supported by GAUK 170/50/75014

RADIOACTIVELY LABELED RAMUCIRUMAB: IN VITRO BINDING AND INTERNALIZATION STUDIES IN VEGFR-2 POSITIVE CELLS

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Ramucirumab (RAM) is clinically used fully humanized monoclonal antibody directed against an extracellular domain of vascular endothelial growth factor receptor 2 (VEGFR2). The VEGFR2 is a key receptor responsible for the angiogenesis and was found to be overexpressed in some types of solid tumors (e.g. gastric, pancreatic, breast or lung cancer). RAM binds the receptor with much greater affinity than its natural ligand and inhibits its function. With proper radiolabeling RAM could be potentially used either as a radiodiagnostic tool for imaging of VEGFR2-positive tumors or in a targeted radiotherapy. The aim of this work was to evaluate the influence of the radiolabeling process on the RAM immunoreactivity.

Several methods of either direct or indirect (via chelating agents) radiolabeling and nuclides (^{99m}Tc, ^{131}I, and ^{67}Ga) were employed in experiments. All prepared radiopharmaceuticals were tested for radiochemical purity and stability using HPLC and ITLC methods and for *in vitro* receptor-ligand binding affinity. Two VEGFR2 expressing human cancer cell lines (PC3, SKOV3) were used in the binding study.

All employed radiolabeling methods were suitable for the RAM labeling. However, significant differences were observed in radiochemical purity, stability and also in the deterioration of the immunoreactivity of the monoclonal antibody after the radiolabeling process. The direct ^{99m}Tc-labeling provided relatively low radiochemical purity, the iodination exhibited low stability and both of these direct labeling methods negatively influenced the immunoreactivity of RAM. All the indirect radiolabeling methods preserved RAM specific binding to the VEGFR2. However, the RAM binding kinetics was slightly altered depending on the radiolabeling conditions.

The study was supported by GAUK(998216/C/2016), SVV(260414) and PROGRES Q42.

STRESS-INDUCED SENESCENCE IN THE HEART– OPTIMALIZATION OF METHODOLOGY

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Stress-induced senescence is specific form of cellular senescence, when mitotic cells exposed to non-lethal stress exhibit senescence-associated secretome phenotype (SASP); state characterized by permanent G1 arrest, alteration of their transcriptome and secretion of specific cytokines etc.. Recently, stress-induced senescence was described also in post-mitotic cells, but its role is unclear. In the heart, cardiac as well as non-cardiac cells could exhibit SASP in response to non-lethal dose of cardiotoxic agents and also in disease state.

Because this phenomenon is of great importance, the main aim of this project is to set up adequate and relevant methodology applicable in our lab for studying the SASP *in vitro* (primary rat cardiomyocytes or H9c2 cardiomyoblasts) and *in vivo*. Furthermore, recently changes in cardiac electrical conduction changes were described, but only on *ex vivo* models. Therefore, secondary aim of this project is to study potential cardiotoxic effects of bisphenols.

Up to date, we have performed pilot experiments with acute administration of bisphenol A in rats to test study design and bisphenol A formulation with different co-solvents. Pilot *in vitro* experiments with using H9c2 cardiomyoblasts and neonatal rat cardiomyocytes were also performed to optimize selected antibody to common cardiokines. Finally, an isolation of adult rat cardiomyocytes using various methods were performed.

The study was supported by the PROGRES Q42 by the Fund of the Dean of FAF UK.

MEMBRANE AND SOLUBLE ENDOGLIN ROLE IN EARLY DEVELOPMENT OF ENDOTHELIAL DYSFUNCTION IN MICE

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Membrane endoglin (Eng) expression is linked to regulation of eNOS in endothelial cells via Smad2/3 signaling resulting in proper function of endothelium. We hypothesized that hypercholesterolemia alters endoglin expression/signaling with respect to endothelial function in aorta before formation of any atherosclerotic changes in mice.

Two-month-old hypercholesterolemic ApoE/LDLR-deficient (ApoE/LDLR$^{-/-}$) female mice and wild type C57BL/6J mice were fed chow diet. Plasma samples were tested for biochemical profile and Luminex analysis. Vascular reactivity was measured by wire myograph and expressions of NO-related markers were assessed by Western blot.

ApoE/LDLR$^{-/-}$ mice demonstrated hypercholesterolemia accompanied by significantly increased levels of sEng, pro-inflammatory P-selectin and a disruption of NO metabolism. Functional data indicated that ApoE/LDLR$^{-/-}$ mice have reduced relaxation capability of smooth muscle cells and activated compensatory mechanism generating NO from eNOS-independent pathway sources. Western blot analysis showed significantly reduced expression of membrane Eng, eNOS and its active form p-eNOS as well as phosphorylated Smad2/3 in ApoE/LDLR$^{-/-}$ mice. The expression of heme oxygenase 1, an enzyme leading to higher NO release, was significantly lower in ApoE/LDLR$^{-/-}$ group. Further, the expression of phosphorylated myosin light chain, a major regulatory component in smooth muscle contraction, was significantly decreased in aorta of ApoE/LDLR$^{-/-}$ mice.

We postulate that the reduced endoglin expression is related to vascular dysfunction in aorta prior formation of atherosclerotic lesion, suggesting an important role of endoglin in cholesterol-induced endothelial/vascular dysfunction.

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ENDOGLIN IS INVOLVED IN CHOLESTEROL-INDUCED ENDOTHELIAL DYSFUNCTION AND MONOCYTE ADHESION IN HUMAN AORTIC ENDOTHELIAL CELLS

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Endoglin (CD105, TGF-βRIII receptor), acts as auxiliary partner protein in TGF-β receptor complex being essential for proper function of endothelium, but might also participate in inflammatory infiltration of leukocytes. We hypothesized that membrane endoglin participates in 7-ketocholesterol (7K) induced development of endothelial dysfunction.

HAECs were exposed to 7K (5, 10μg/mL) for 12 hours. Gene expression (endoglin, KLF6, RELA (NF-κB p65), NR1H3 (LXR), and ICAM-1) was evaluated using qRT-PCR. Protein levels of endoglin, ICAM-1 and Selectins were evaluated by immunofluorescence flow cytometry analysis and intracellular localization of RELA, eNOS, and p-eNOS was evaluated using confocal fluorescent microscopy.

Gene expression and protein levels of endoglin, eNOS, p-eNOS and cell adhesion molecules (ICAM-1, E/P-selectin) were significantly increased after 12h premedication with 7K compared to non-treated cells. KLF6, RELA and NR1H3 transcription genes regulating endoglin expression were increased after 12h premedication with 7K in dose 10μg/mL. Inhibition of either results in inhibition of 7K induced increase of endoglin expression. 7K was able to increase adhesion and transmigration of THP-1 monocytes, trough endothelial cells monolayer (HAECs). Silencing of endoglin in HAECs by siENG inhibited adhesion and transmigration of THP-1 monocytes trough endothelial monolayer.

In this study, we demonstrated that 7K is able to induce inflammation and increase endoglin expression in endothelial cells via KLF6, RELA and NR1H3 transcription genes. We also demonstrated that 7K induced adhesion and transmigration of monocytes trough endothelial monolayer depends on the expression of endoglin suggesting that endoglin might play crucial role in cholesterol (oxysterol) induced endothelial dysfunction.

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THE ANTIPLATELET EFFECT OF 4-METHYLCATECHOL WAS CONFIRMED ON IN VIVO (EX OVO) HEN´S EGG TEST ON THE CHICK AREA VASCULOSA

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The flavonoid metabolites, produced by human colon bacteria, are derivatives of benzoic, propionic, acetic acids and hydroxybenzenes, e.g. 4-methylcatechol (4-MC), pyrogallol, phloroglucinol or resorcinol. These metabolites reach in plasma higher concentrations than parent flavonoids[1] and could be responsible for their observed positive effects.

The antiplatelet research of selected metabolites showed that all four hydroxybenzene derivatives appeared to have a biologically relevant effect; moreover 4-MC was even 10x times more active on arachidonic acid induced aggregation than clinically used acetylsalicylic acid (ASA). The mechanisms of action of this compound include inhibition of platelet serotonin release and partly thromboxane A_2 synthase inhibition.

To confirm this positive antiplatelet effect in a more biological system, a shell less hen's egg test on the chick area vasculosa was used. Here ASA pretreatment decreased the lethality after induction of aggregation from 46% to 7% after the first hour and from 60% to 27% after 24 hours. 4-MC afforded even more protection; there was no mortality after the first hour and only 7% lethality after 24 hours, which corresponded with the negative control. Moreover ASA and 4-MC showed significant improvement of the size and intensity of thrombosis in comparison to negative control.

This study was supported by EFSA-CDN (No.CZ.021.01/0.0/0.0/16_019/0000841) co-funded by ERDF, PROGRES (170/11/1108-2), Charles University (SVV 260 414) and German Research Foundation (CRC 1278-PolyTarget).

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DEVELOPMENT OF NOVEL DISINFECTANTS AGAINST PATHOGENS OCCURING IN THE HOSPITAL ENVIRONMENT

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In the project, we have prepared 64 novel compounds with a different length of alkyl chain (C8, C10, C12, C14, and C16) based on quaternary ammonium salts. The newly synthesized compounds were tested against aerobic bacteria (Gram-positive and Gram-negative), anaerobic bacterial strain *Clostridium difficile*, yeasts and filamentous fungi, Varicella zoster virus (VZV) and green algae *Pseudokirchneriella subcapitata*. The project is designed for development of various (3-6) mixtures with strong disinfecting properties and wide spectrum of efficacy by combining individual agents with more specific efficacy. The most promising agents against aerobic bacteria were 31-C16, 32-C16, 27-C14, 20-C16 and 18-C16. Against *Clostridium difficile*, the highest efficacy showed compounds 33-C12 and 32-C14. The highest efficacy against yeasts was observed for 32-C16, 18-C16, 16-C16 and 32-C14, against filamentous fungi then for 18-C16 and 16-C16. Although, the efficacy on viruses in quaternary ammonium salts is relatively rare, 32-C14 achieved $\log_{10}$ reduction factor of 5 in the virus titre after 5-minute exposure. A little less effective was 28-C8 and 33-C14. Supplemental efficacy against green algae was very high for 32-C16, 27-C14, 18-C16, 16-C16 and 27-C12. Based on *in vitro* testing, the most effective substances were selected and four mixtures have been formulated. The mixture 4 showed the results at least comparable to AJATIN.

The study was supported by the Ministry of Health of the Czech Republic (project NV18-09-00181 and 15-31847A) and by the Ministry of Education, Youth and Sports of the Czech Republic (student grant SV/FVZ201607).

EFFECT OF 4-METHYLCATECHOL ON THE RELEASE OF SEROTONIN AND THROMBOXANE IN HUMAN PLATELETS

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Platelets are crucial for blood coagulation, which preserves the integrity of the cardiovascular system. However, dysregulation of their function may lead to many diseases, in particular stroke and acute myocardial infarction, which are the most prevalent causes of mortality in developed countries[1]. Current antiplatelet therapy has many disadvantages, including lack of efficiency, side effects, or cost[2, 3]. Flavonoids, and in particular their metabolites, seem to possess antiplatelet potential. The aim of the study was to continue in the assessment of the mechanism of action of 4-methylcatechol (4-MC), a known colonic metabolite of flavonoids. Both serotonin (5-HT) and thromboxane A_2 (TXA_2) are important factors involved in formation of a blood clot. The effect of 4-methylcatechol (4-MC) on the release of these 2 agents from platelets was determined. The amount of released substances after addition of an aggregation inducer was measured by corresponding ELISA assay kits. 4-MC affected the release of 5-HT from platelets, but had at μM concentrations no effect on the release of TXA_2 or its concentration inside the platelets. 4-MC does not inhibit TXA_2 release from platelets, hence the major antiplatelet mechanism of action of 4-MC is currently unknown and further investigation is needed.

The study was supported by PROGRES (170/11/1108-2) and Charles University (SVV 260 414).

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BIOCHANIN A AND GLYCITEIN INDUCE AN ENDOTHELIUM-INDEPENDENT DILATION OF RAT AORTA AND PORCINE CORONARY ARTERY.

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Flavonoid intake seems to be inversely associated with mortality on coronary heart disease. In particular the consumption of isoflavonoids is increasing in general population, due to the use of food supplements and a variety of soy products, legumes and nuts.

In the framework of this study, fifteen isoflavonoids were screened *ex vivo* for their vasorelaxant properties in norepinephrine pre-contracted rat aorta. Among them, biochanin A and glycitein were selected for additional experiments on isolated porcine coronary arteries and mechanistic studies. Both of them exhibited an endothelium independent relaxation of the rat aortic and porcine coronary vasculature-*ex vivo*. For further investigation of the possible mechanism of action, the coronary arteries were incubated in the presence of the tested isoflavonoid (concentrations ranging from 3 to 30 μM), and then cumulatively contracted by KCl, $CaCl_2$, serotonin or U46619. Vasoconstriction produced by one or more of these stimuli was inhibited (at least partially) by both tested compounds in a dose-dependent manner, indicating a direct vasorelaxant effect on the arterial smooth muscles. Biochanin A was found to be more potent, achieving a lower EC_{50} and blocking the effect of all four constrictors. These results suggest a positive impact of the isoflavonoids on some cardiovascular pathologies, that needs to be further confirmed by *in vivo* studies.

The study was supported by the Charles University (grant No. 1080217).

COPPER CHELATORS AND THEIR ABILITY TO BIND ZINC

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Our knowledge about the role of zinc in the human organism has been constantly expanding. It is well know that zinc has many physiological roles and is essential for many enzymes and transcription factors. Its deficiency manifests by many symptoms ranging from immune dysfunction, growth retardation to skin disorders and is mostly caused by insufficient zinc intake in the diet or derangement in GIT absorption. Less is known, that zinc deficiency can follow long-life treatment by metal chelators, which are mostly non-selective.

Hence, the aim of this work was to study possible ability of clinically and experimentally used copper chelators to chelate zinc ions by using a competitive spectrophotometric method based on dithizone. The tested compounds included D-penicillamine, trientine and ammonium tetrathiomolybdate /ATTM/ which have been clinically used or tested in the treatment of Wilson's disease, and experimentally tested series of four 8-quinolinoles (8-quinolinol, 5-chloro-7-iodo-8-quinolinol, 5,7-dichloro-8-quinolinol /chloroxine/ and 5-nitro-8-quinolinol /nitroxoline/). Various physiologically relevant pH levels ranging from 4.5 to 7.5 were simulated.

The ability to bind zinc was observed in all of the tested compounds. The most potent clinically used chelator was trientine with approximately 65% zinc binding activity in the molar ratio 1:1 at pH 7.5. However, experimentally used 8-quinolinoles showed proportional or even higher binding capacity at lower pH levels. Especially nitroxoline was a very potent zinc chelator at all pH levels. Surprisingly all of the tested compounds showed higher affinity for Zn ion in comparison with ability of D-penicillamine to bind Cu ions.

In conclusion we can assume that clinically used copper-chelators as well as ATTM and 8-quinolinoles are also relatively potent zinc chelators and hence their longer administration use can possibly result in adverse effects associated with zinc deficiency.

The study was supported by Charles University (SVV 260 414)

Thursday 24. January 2019

Lecture hall 2463

Session 7 - Pharmaceutical Technology: ***8:15 – 10:00***

THE USE OF GRAVITATIONAL CONSOLIDATION FOR THE PREDICTION OF ANGLE OF INTERNAL FRICTION

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On the particle-particle basis, the angle of internal friction (*AIF*) commonly belongs to important material parameters characterizing behaviour of moving particles. The *AIF* is usually determined by measuring in a shear cell but a lot of time and a high amount of tested material are main disadvantages.[1] Thus, the purpose of this study was to find out if the gravitational consolidation could be used for the estimation of the *AIF*.

Seven types of a lactose powder were consolidated under a controlled number of taps. The correlation between the powder porosity and the number of taps were used for the factor porosity determination. From the arcus tangent of the slope of the linear relationship between the porosity factor and the number of taps, the *AIF* was estimated [2,3] and compared with that of determined by measurement using the Jenike shear cell.

A good correlation between the AIF estimated from the gravitational tapping and the AIF determined by Jenike shear cell was observed in this study. The prediction of the AIF by simple gravitational tapping would be useful as tapping belongs to standard methods of estimating of tapped density and Hausner ratio used in pharmaceutical industry. However, more research in this area using different materials is necessary.

The study was supported by the Funding Agency of Charles University under Grant No. 1286218/2018 and by SVV 260 401.

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BIODEGRADABLE POLYMERIC NANOPARTICLES FOR MEDICAL APPLICATIONS

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Our work is focused on polymeric nanoparticles formulation and characterization with intent of achieve prolonged response, and to reduce adverse effects of selected incorporated drugs (terbinafine hydrochloride, rifampicin, oligomer). We used originally synthesized and fully biodegradable, linear or branched polyesters based on poly(lactic-co-glycolic acid). Branched ones are synthesized with the addition of the branching unit such as poly(acrylic acid), mannitol, or erythritol derivatives. Nanoparticles were prepared using double-emulsion method, or more frequently by modified nanoprecipitation method.[1] We monitored multiple parameters (particles size, polydispersity, zeta potential) using Zetasizer Nano ZS by Malvern. The size of the nanoparticles were 100-600 nm, and could be modify by the choice of the polyester and its concentration, and mixing technique of phases. For analysis, we used HPLC, fluorometer, or spectrophotometer, according to the analyzed drug. Dissolution tests showed prolonged release of incorporated drugs, which we attribute to the gradual swelling and degradation of the polyester in an aqueous medium.[2] The release of the incorporated drug is pH dependent, and it can be accelerated by lowering the pH value of the dissolution medium. Examined polyesters, especially the branched ones, are perspective, original, and suitable for further observation.

The study was supported by SVV 260 401.

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THE PROPERTIES OF CHITOSAN AS A DRUG CARRIER

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Chitosan is nowadays one out of the most investigated excipients used in many pharmaceutical formulations including gels, microspheres, coatings for liposomes, and tablets for its inherent properties such as biodegradability, low toxicity and good biocompatibility[1]. It is a polysaccharide comprising copolymers of glucosamine and *N*-acetylglucosamine, prepared by hydrolysis of chitin by using several alkaline treatment.[2] The aim of the work is to investigate the properties of chitosan as a drug carrier for the further use as a hydrophilic filler to improve drug solubility in interactive mixtures or in matrix colon-targeted delivery systems. Different methods such as optical and scanning electron microscopy, laser diffraction, differential scanning calorimetry, and a shear cell measurement (Freeman powder rheometer) were used to characterize the substance. The particles were irregular shape and rough surface (pD_F = 1,045, shape factor = 0,56) having the median particle diameter x_{50} = 88,8 μm and span 1,93. Shear cell measurement showed that chitosan has poor flowability. These initial measurements were used to characterize particle properties of chitosan, however, flow and compaction properties will be further investigated.

The study was supported by the Funding Agency of Charles University under Grant No. 1286218/2018 and by SVV 260 401.

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A STUDY OF THE DRUG RELEASE FROM MATRIX TABLETS WITH POLYVINYL ALCOHOL

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The aim of this study was to determine the effect of the different retardant concentration on drug release from hydrophilic matrix tablets. At the same time, the influence of the type of dry binder used was investigated. Polyvinyl alcohol was used as the retarding agent at the concentrations of 30, 40 and 50 %. α-lactose monohydrate and microcrystalline cellulose in the ratio of 3:1 in the physical mixture and in MicroceLac® 100 were used as dry binders. MicroceLac® 100 is a coprocessed dry binder. Salicylic acid was used as a model less soluble drug. The tablets were prepared by direct compression method. Dissolution testing was performed using the rotating basket method. The results of the dissolution test were evaluated by nonlinear regression analysis

Increasing additions of polyvinyl alcohol decreased drug release rate. It has been found that the type of dry binder does not affect neither the mechanism nor the release rate of salicylic acid. The dissolution behavior of tablets, which contained the physical mixture or the coprocessed dry binder and the same amount of polyvinyl alcohol was comparable.

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ORAL DELIVERY OF OLIGONUCLEOTIDES FOR LOCAL TREATMENT OF INFLAMMATORY BOWEL DISEASE

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Inflammatory bowel disease (IBD) describes pathological conditions characterised by inappropriate and sustained activation of the mucosal immune system of the small intestine and/or colon.[1] Local treatment is preferred over systematic delivery often accompanied with undesired side effects. In IBD, cationic peptides are expressed in the area and phagocytic immune cells infiltrate the site of inflammation.[2] By delivery of an anti-inflammatory acting miRNA oligonucleotide, production of pro-inflammatory cytokines by these immune cells decreases, and the inflammatory process itself is suppressed. However, delivery of instable negatively charged macromolecular oligonucleotides represents a challenge.[3] Self-nanoemulsifying drug delivery system (SNEDDS) has been utilised to deliver a hydrophobic complex of oligonucleotides orally.[4]

The complexes were prepared from a model 20-nucleotide long oligomer and a cationic lipid dimethyldioctadecylammonium bromide (DDAB) or 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP) with yields over 95% for molar ratio 1:60. The size of the complexes was estimated by atomic force microscopy to be 75-127 nm and 33- 62 nm, for DOTAP and DDAB complex respectively. The size of dispersed loaded SNEDDSs, ~200 nm, and negative surface charge enabling passive targeting make the formulation suitable for intended purpose.

The study was supported by SVV 260 401 and Czech science foundation 270/53/75302.

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NOVEL FORMULATIONS FOR TOPICAL APPLICATION OF IMIQUIMOD

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Imiquimod (IMQ) is a heterocyclic imidazoquinoline used in the treatment of various viral or neoplastic skin diseases.[1] Aim of this study was to prepare suspensions, emulsions and liposomes for dermal administration of 1% IMQ and evaluate its *in vitro* permeation on human skin. IMQ is commercially available as a cream under the brand name Aldara® (5% IMQ). This formulation possesses undesired disadvantages like low skin permeation, instability and side effects on skin.[2] In order to overcome these obstacles, these formulations with lower concentration of IMQ and different permeation enhancers (L-Pro2, DDAK and oleic acid) were prepared. *In vitro* permeation experiments were carried out in Franz Diffusion Cells on human skin while Aldara® was used as a control. After 8 and 24 hours the concentration of IMQ in each skin layer, as well as in acceptor phase (Phosphate buffer, pH 7.4), was determined. In emulsions and suspensions a higher concentration of IMQ was proved in epidermis and lower concentration in the deeper compartments compared to Aldara®. The liposomal formulations did not show such high effect to deliver IMQ to the epidermis. In the future, optimized liposomal formulations will be developed for better potential delivery of IMQ.

The study was supported by the Czech Science Foundation (13-23891S) and the Charles University in Prague (GAUK 184217 and SVV 260 401).

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LIPOSOMES FOR DERMAL DRUG DELIVERY

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Over the past years, (trans)dermal delivery of biologically active molecules has become very popular due to its advantages over other routes of administration.[1] Different methods and vehicles have been used to improve dermal delivery, of which, liposomes seem to be the most studied.[2] Recently, our research group prepared Imiquimod-containing liposomal mixtures which were unable to efficiently deliver Imiquimod to epidermis in higher concentrations than the commercially available cream (Aldara). Inspired by the previous results, the aim of the present study is, to prepare Imiquimod loaded liposomes following an alternative approach and evaluate their ability to deliver the active substance to the skin layers.

The study was supported by SVV: 260401 and Czech Science Foundation (GACR 19-09600S)

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