FACULTY OF PHARMACY
IN HRADEC KRÁLOVÉ
Charles University

8th Postgraduate
and
6th Postdoc Conference

Book of Abstracts

24th and 25th January 2018

This conference is supported by the STARSS project co-funded by the European Union.
Reg. No. CZ.02.1.01/0.0/0.0/15_003/0000465

EUROPEAN UNION
European Structural and Investment Funds
Operational Programme Research,
Development and Education

MINISTRY OF EDUCATION,
YOUTH AND SPORTS

CONTENT

This conference is supported by the STARSS project co-funded by the European Union.
Reg. No. CZ.02.1.01/0.0/0.0/15_003/0000465

EUROPEAN UNION
European Structural and Investment Funds
Operational Programme Research,
Development and Education

MINISTRY OF EDUCATION,
YOUTH AND SPORTS

Bioorganic and Pharmaceutical chemistry

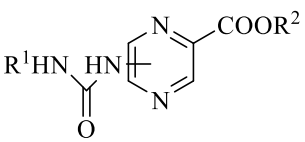
PYRAZINAMIDE; NOT THE END OF THE STORY.

BOUZ, G., ZITKO, J., DOLEŽAL, M.

Department of Pharmaceutical Chemistry and Drug Analysis, Faculty of Pharmacy in Hradec Kralove, Charles University, Czech Republic

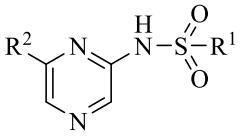
e-mail: bouzg@faf.cuni.cz

Despite being an old disease, tuberculosis remains the leading cause of death from infectious diseases at present time[1]. Among anti-tuberculars, pyrazinamide particularly has captured research attention. Several new specific mechanisms have been recently identified by which pyrazinamide exerts its antimycobacterial effect. This achievement opened a window for possible structural modifications in order to improve its biological activity and overcome emerging resistance. We will discuss two derivatization approaches of pyrazinamide. In the first series **(1)**, urea moiety was introduced to the pyrazine core. Among all prepared compounds, propyl 5-(3-phenylureido)pyrazine-2-carboxylate (MIC_{Mtb} = 1.56 μg/mL, 5.19 μM) and propyl 5-(3-(4-methoxyphenyl)ureido)pyrazine-2-carboxylate (MIC_{Mtb} = 6.25 μg/mL, 18.91 μM) had high antimycobacterial activity against *Mtb* H37Rv with no *in vitro* cytotoxicity on HepG2 cell line up to the highest tested concentrations[2]. In the second series **(2)**, different pyrazine sulfonamides were prepared. Synthesized compounds are being evaluated for their biological activities, including anti-infective and any possible anti-cancer properties. Obtained results will be discussed in the presentation.



R^1: Alkyl/Aryl Substituents
R^2: H/C_3H_5

(1)



R^1: Aryl Substituents
R^2: H/Cl

(2)

The study was supported by the Ministry of Education, Youth and Sports of the Czech Republic (SVV 260 401) and (SVV 260 416), as well as by Grant Agency of Charles University (projectC-C3/1572317) and Czech Science Foundation (project No. 17-27514Y).

References

1. World Health Organization, Global Tuberculosis, Report 2017. WHO/HTM/TB/2017.23.
2. BOUZ, G., JUHÁS, M., NIKLOVÁ, P., JAND´OUREK, O., PATEROVÁ, P., JANOUŠEK, J., TŮMOVÁ, L., KOVALÍKOVÁ, Z., KASTNER, P., DOLEŽAL, M., ZITKO, J.: Molecules, 22, 2017, 1797.

PREPARATION OF BENZODIAZINES WITH BRONCHODILATORY ACTIVITY

BROŽOVÁ, Z. R,[1] POUROVÁ, J.,[2] VOPRŠÁLOVA, M,[2] ŠPULÁK M.[1]

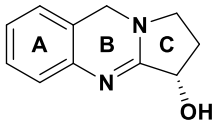
[1] Department of Organic and Bioorganic Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

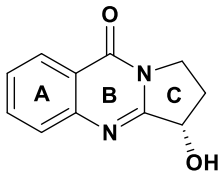
[2] Department of Pharmacology and Toxicology, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

e-mail: hruskovz@faf.cuni.cz

Asthma bronchiale, a chronic inflammatory disease, is becoming increasingly prevalent in most developed and many developing countries. Natural products have historically been an excellent source of new drugs for the pharmaceutical industry.

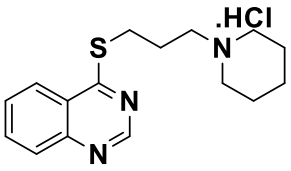
(-)Vasicine (**1**) and (-)vasicinone (**2**) are major alkaloids isolated from *Justicia adhatoda* L. known to have a moderate bronchodilatory effect.

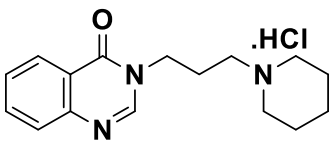


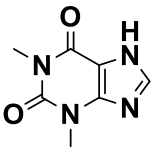


1 ED_{50} = 0,323 mmol/L **2** ED_{50} = 1,3 mmol/L

Several literature reports have described the modifications of the (-)vasicinone structure and showed that ring C is not necessary for a compound to possess bronchodilatory effect. So far, we have synthesized the first series of derivatives[1], where the C-ring was replaced with alkyl chain terminated by a tertiary amine. The most active derivatives (**3, 4**) displayed bronchodilatory activity far exceeding the effect of theophylline (**5**) as a standard drug on isolated rat trachea.

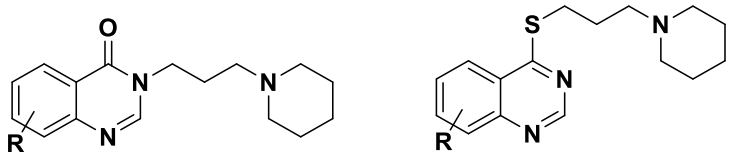






3 ED_{50} = 3,42 µmol/L **4** ED_{50} = 28,3 µmol/L **5** ED_{50} = 2090 µmol/L

Structures **3** and **4** were further modified, and another series of derivatives with substitution on ring A was synthesized and their bronchodilatory activity was evaluated.



R: -F, -diF, -Cl, -diCl, -Br, -OCH_3, -diOCH_3,

The study was supported by Charles University in Prague (GAUK 398 315), SVV-260-401 and Czech Science Foundation (15-07332S).

REFERENCES:

1. ŠPULÁK, M., POUROVÁ, J., VOPRŠÁLOVÁ, M., MIKUŠEK, J., KUNEŠ, J., VACEK, J., GHAVRE, M., GATHERGOOD, N., J., POUR, M.: *EUR. J. MED. CHEM*. 74, **2014**, 65-72.

INTRAMOLECULAR TSUJI-TROST REACTION: NEW ROUTE TO HIGHLY SUBSTITUTED PYRANONES

BRŮŽA, Z.,[1] KRATOCHVÍL, J,[1] POUR, M.[1]

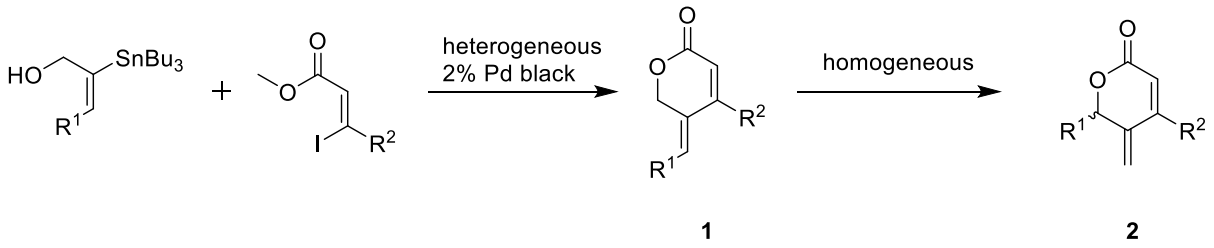
[1]Charles University, Faculty of Pharmacy in Hradec Kralove, Hradec Kralove, 500 05, Czech Republic

Email: bruzazb@faf.cuni.cz

Lactones (Scheme 1, **1**) prepared by from β–iodoacrylic esters and 2-tributylstannyl allyl alcohols under heterogeneous catalysis[1] conditions can isomerize to 5-methylene pyranones (Scheme 1, **2**). Interestingly homogeneous catalysis is required to trigger the isomerization of pyranones **1**.

Following extensive optimization, a library of 18 compounds was obtained.

Since the reaction generates a new stereogenic center, chiral phosphines were employed to investigate the possibility of developing an enantioselective process.



1 **2**

Scheme 1. Preparation of compounds

The study was supported by Grant Agency of Charles University (project No. 1054216), Czech Science Foundation (project No. 15-07332S) and Faculty of Pharmacy in Hradec Kralove (SVV-260-401).

References

1. J. KRATOCHVIL, Z. NOVAK, M. GHAVRE, L. NOVAKOVÁ, A. RUZICKA, J. KUNEŠ, M. POUR, Org. Lett., 2015, 17, 520-523.

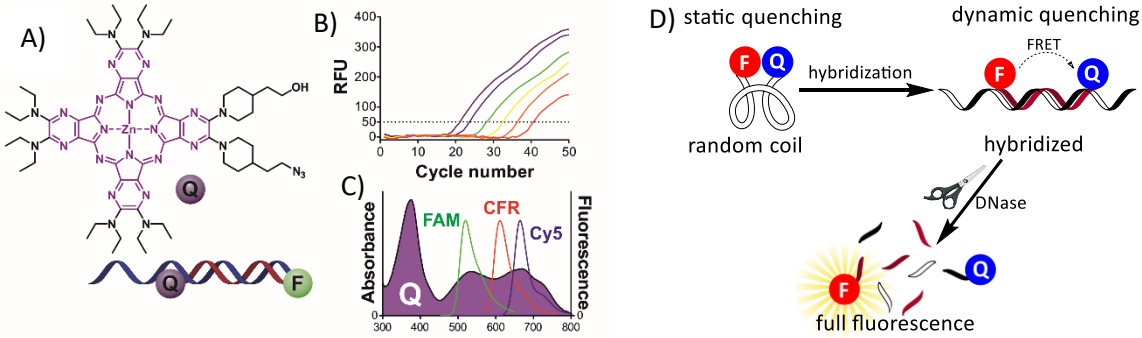
NEW AZAPHTHALOCYANINE DARK QUENCHER USABLE IN POLYMERASE CHAIN REACTION

DEMUTH, J.,[1] KUČERA, R.,[1] ZIMČÍK, P.,[1] MILETÍN, M.,[1] NOVÁKOVÁ, V.,[2]

[1] Department of Pharmaceutical Chemistry and Pharmaceutical Analysis, [2] Department of Biophysics and Physical Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

e-mail: demuthj1@faf.cuni.cz

Unsymmetrical dialkylamino substituted azaphthalocyanines (AzaPc, Fig. A) have unique photophysical properties – e.g. light absorption between 300 and 700 nm (Fig. C), almost no self-fluorescence and ability to quench fluorescence of other compounds. This makes AzaPcs suitable candidates for universal dark quenchers.[1,2] To confirm the assumptions, series of oligodeoxyribonucleotide double-labeled probes were prepared with this AzaPc at 3′-end and with commercial fluorophores at 5′-end. Fluorophores were chosen with an emphasis to cover whole emission spectrum of fluorophores, which are used in polymerase chain reaction (PCR), *i.e.* fluorescein, CAL Fluor Red 610 and Cy5. A model of TaqMan assay was developed to enable direct photophysical comparison of different quenchers (Fig. D). AzaPc quencher had remarkably higher quenching efficiency in this hybridization assay than commercially used dark quencher such as BHQ-1, BHQ-2, BBQ-650. The real application (quantitative PCR) was tested for quantification of SLCO2B1 transporter gene (Fig. B). Resulting calibration curves indicated linearity in range from 10^2 to 10^7 of target copies.



The study was supported by Grant Agency of Charles University (1168217) and SVV 260 401.

References

1. KOPECKY, K.; NOVAKOVA, V.; MILETIN, M.; KUCERA, R.; ZIMCIK, P.: *Tetrahedron*, 67 (33), 2011, 5956-5963.
2. KOPECKY, K.; NOVAKOVA, V.; MILETIN, M.; KUCERA, R.; ZIMCIK, P.: *Bioconjugate Chem.*, 21 (10), 2010, 1872-1879.

THE NOVEL LEAD CANDIDATES FOR THE TREATMENT OF ORGANOPHOSHOROUS INTOXICATION

GORECKI, L.,[1,2] KORABECNY, J.,[1,2] MALINAK, D.,[2] HEPNAROVA, V., [1,2] HRABINOVA, M.,[1,2] SOUKUP, O.,[1,2] JUN, D., [1,2] MUSILEK, K.,[1,2] KUCA, K.,[1,2]

[1] Department of Toxicology and Military Pharmacy, Faculty of Military Health Sciences, University of Defence, Trebesska 1575, 500 01 Hradec Kralove, Czech Republic

[2] Biomedical Research Centre, University Hospital Hradec Kralove, Sokolska 581, 500 05 Hradec Kralove, Czech Republic

e-mail: lukasgorecki@seznam.cz

Mono- and bis-pyridinium aldoximes are the only causal antidotes that are designated for the treatment of organophosphate poisoning (OP). Intoxication by OP is caused either by pesticides or by the nerve agents, the latter belong to group of chemical warfare agents. These compounds irreversibly inhibit essential enzyme acetylcholinesterase that is no more able to fulfill its physiological function. Mono- and bis-pyridinium aldoximes are able to cleave organophosphate acetylcholinesterase bond and restore enzyme's catalytic activity. The reactivating ability of aldoximes is hampered by several drawbacks like low blood-brain barrier permeation, low reactivation potency against specific nerve agents etc. In order to obtain efficient treatment of OP, the introduction of novel AChE reactivators is matter of importance. For over 60 years of intensive research, none of the new compounds reached sufficient activity. Herein, we present novel mono quaternary reactivators that abound with excellent in vitro activity to recover AChE activity after intoxication with different nerve agents as well as pesticides. The molecular docking simulations, total synthesis and biological evaluation will be discussed.

The study was supported by specific research University of Defence (SV/FVZ201601) and MH CZ—DRO (University Hospital Hradec Kralove, No. 17-32801A).

NONTOXIC COMBRETAFURANONE ANALOGUES WITH HIGH IN VITRO ANTIBACTERIAL ACTIVITY

HORKÝ, P.,[1] VORÁČOVÁ, M.,[1] SEDLÁK, D.,[2] POUR, M.,[1]

[1] Department of Bioorganic and Organic Chemistry, Faculty of Pharmacy, Charles University, Heyrovského 1203, 500 05 Hradec Králové, Czech Republic

[2] CZ-OPENSCREEN: National Infrastructure for Chemical Biology, Institute of Molecular Genetics, Czech Academy of Sciences, Vídeňská 1083, 142 20 Praha 4, Czech Republic

e-mail: horkyp@faf.cuni.cz

A library of thirty two 3,4-diphenylfuranones related to both combretastatin A-4 and antifungal 5-(acyloxymethyl)-3-(halophenyl)-2,5-dihydrofuran-2-ones was prepared. Cytotoxic effects on a panel of cancer and normal cell lines and antiinfective activity were evaluated, and the data were complemented with tests for the activation of caspase 3 and 7. High cytotoxicity was observed in some of the halogenated analogues, eg. 3-(3,4-dichlorophenyl)-4-(4-methylphenyl)-2,5-dihydrofuran-2-one with IC_{50} 0.12 e0.23 mM, but the compounds were also highly toxic against non-malignant control cells. More importantly, notable antibacterial activity indicating Gþ selectivity has been found in the 3,4-diarylfuranone class of compounds for the first time. Hydroxymethylation of furanone C5 knocked out cytotoxic effects (up to 40 mM) while maintaining significant activity against Staphylococcus strains in some derivatives. MIC95 of the most promising compound, 3-(4-bromophenyl)-5,5-bis(hydroxymethyl)-4-(4-methylphenyl)-2,5-dihydrofuran-2-one against S. aureus strain ATCC 6538 was 0.98 mM (0.38 mg/mL) and 3.9 mM (1.52 mg/mL) after 24 and 48 h, respectively.[1]

This study was supported by the Czech Science Foundation (project No.15-07332S), from Charles University (projects No.1906214 and SVV 260 401) and from Ministry of Education, Youth and Sports of the Czech Republic (LO1220 and LM2015063)

References

1. HORKÝ, P., VORÁČOVÁ, M., KONEČNÁ, K., SEDLÁK, D., BARTŮNĚK, P., VACEK, J., POUR, M.: Eur. J. Med. Chem, 143, 2018, 843-853

SYNTHESIS OF NEW SULFONATED AZAPHTALOCYANINE AND ITS PHOTOPHYSICAL AND PHOTODYNAMIC PROPERTIES

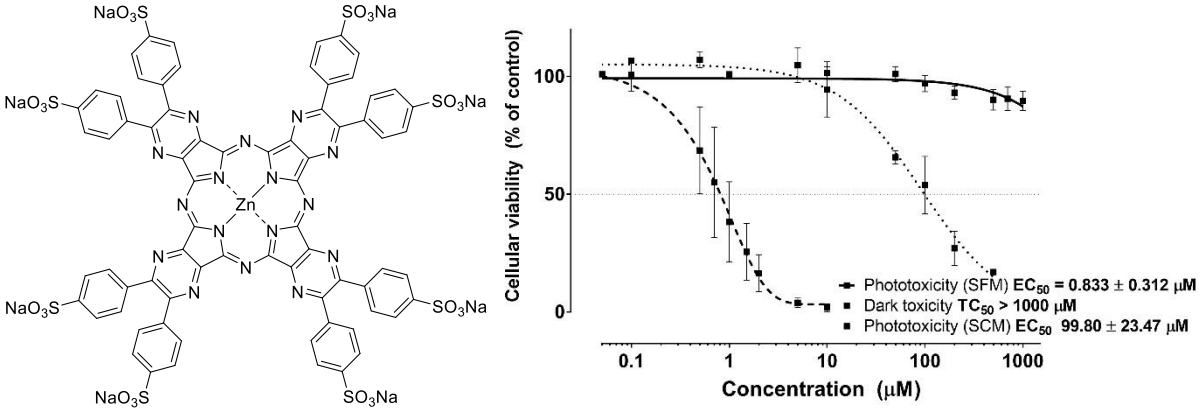
KOLLAR, J.[1], JANCAROVA, A.[1], MACHACEK, M.[2], ZIMCIK P.[1]

[1] Department of Pharmaceutical Chemistry and Pharmaceutical Analysis, [2] Department of Biochemical Sciences, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

e-mail: kollarj@faf.cuni.cz

Azaphthalocyanines (AzaPc) are a group of organic dyes with promising photophysical properties (strong absorption in area over 650 nm and strong singlet oxygen production) to be used as photosensitizers in PDT. The main drawbacks are, however, their low water solubility and strong tendency to aggregation that decrease their photodynamic activity.

The aim of this work was to synthesize an anionic derivative of AzaPc substituted with sulfonic groups on periphery which is characterised by good solubility in water and to evaluate its photodynamic properties. The first step in synthesis was condensation of diaminomaleonitrile and benzil giving 5,6-diphenylpyrazine-2,3-dicarbonitrile. Subsequently, the cyclotetramerisation with zinc acetate using 2-dimethylaminoethanol as a solvent was performed. The final product was obtained by sulfonation with chlorosulfonic acid followed by hydrolysis with sodium bicarbonate. The green coloured product was then purified by size-exclusion chromatography using Superdex® as stationary phase. Sulfonated AzaPc is soluble in water but according to absorption spectra it is partially aggregated. The final AzaPc was tested on photodynamic activity *in vitro* against HeLa cells using serum-free medium (phototoxicity EC_{50} = 0.833 ± 0.312 μM) and was characterized also by low dark toxicity TC_{50} > 1000 μM, HeLa). It was practically inactive in serum-containing medium due to its strong binding to serum proteins. Photophysical properties of binding to serum proteins was studied.



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

RHODANINE DERIVATIVES AS POTENTIAL AGENTS IN TREATMENT OF CHRONIC DIABETIC COMPLICATIONS

KUČEROVÁ-CHLUPÁČOVÁ, M., [1] ŠOLTÉSOVÁ PRNOVÁ, M., [2] MÁJEKOVÁ, M., [2] ŠTEFEK, M.[2]

[1] Department of of Pharmaceutical Chemistry and Pharmaceutical Analysis, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

[2] Department of Biochemical Pharmacology, Institute of Experimental Pharmacology & Toxicology, Slovak Academy of Sciences, Slovak Republic

e-mail: kucerom@faf.cuni.cz

In our continuous efforts to find new aldose reductase inhibitors,[1] a series of [(5*Z*)-(5-arylalkylidene-4-oxo-2-thioxo-1,3thiazolidin-3yl)]acetic acids[2] was tested on inhibition of aldose reductase (ALR2). The enzyme plays a crucial role in the development of long-term diabetic complications.[3] Inhibitory activity of the compounds was determined on ALR2 isolated from rat eye lenses and compared to that of clinically used ALR2 inhibitor epalrestat. Most of the compounds have shown IC_{50} in the submicromolar range and in addition, lower than epalrestat. Inhibitory activity on aldehyde reductase (ALR1) from rat kidneys has been measured in order to determine the selectivity. The obtained results were comparable to the selectivity index of epalrestat. The most potent inhibitor of ALR2 from the series showed mixed type of inhibition. Relationships between chemical structure, lipophilicity and biological activity have been derived. The work has been accompanied by a molecular docking study on 4JIR elucidating the intermolecular interactions.

The study was supported by the research program Development and Study of Drugs Progres Q42 (Charles University, Czech Republic).

References

1. STEFEK, M., SOLTESOVA PRNOVA, M., MAJEKOVA. M., RECHLIN., C., HEINE, A., KLEBE, G.: J. Med. Chem., 58, 2015, 2649–2657.
2. KUCEROVA-CHLUPACOVA, M., DOSEDEL, M., KUNES, J., SOLTESOVA PRNOVA, M., MAJEKOVA, M., STEFEK, M.: Mon. Chem. (accepted).
3. DOLEZEL, J., HIRSOVA, P., OPLETALOVA, V., DOHNAL, J., VEJSOVA, M., KUNES, J., JAMPILEK, J.: Molecules, 14, 2009, 4197–4212.

APPROACHES TO TOTAL SYNTHESIS OF NOSTOTREBIN 6

MATOUŠ P., VORÁČOVÁ M., KUNEŠ J., POUR M.

Department of Organic and Bioorganic Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

e-mail: matousp1@faf.cuni.cz

Nostotrebin 6 (Figure I) is a polyphenolic secondary metabolite containing the cyclopentenedione moiety isolated from cynobacteria *Nostoc* sp. The compound possesses an antimicrobial activity and is also an efficient inhibitor of both acetylcholinesterase and butyrylcholinesterase.[1] No total synthesis has not been reported to date. Possible synthetic approaches towards key intermediates and derivatives will be discussed.

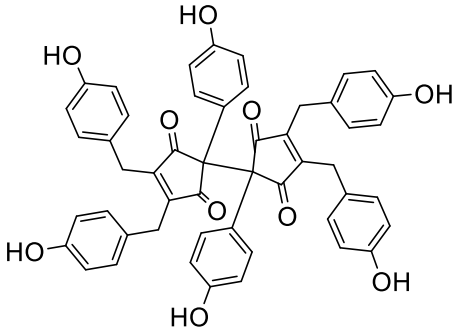


Figure I: Nostotrebin 6

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

References

1. ZELIK, P.; LUKESOVA, A.; CEJKA, J.; BUDESINSKY, M.; HAVLICEK, V.; CEGAN, A.; KOPECKY, J.: J Enzyme Inhib Med Chem, 25 (3), 2010, 414-20.

FIGHTING MICROBIAL RESISTANCE – WHO IS GOING TO WIN?

JANDOUREK, O., KONECNA, K.

Department of Biological and Medical Sciences, Faculty of Pharmacy in Hradec Kralove, Charles University, Czech Republic

e-mail: jando6aa@faf.cuni.cz

Antimicrobial resistance is one of the most threatening as well as widespread global health problems. There can be found at least one resistant microbial strain in every country all over the world. It does not matter if you live in industrial country or developing country, or if you were born either in hot Sahara or beyond polar circle. Microbes are not choosy and we need to find the way how to be more successful in this never-ending fight.

One way how to reach this goal is to find new compounds with novel mechanism of action, better pharmacological properties or simply new molecule that can kill these superbugs without killing us.

It is not only important to design and prepare new molecules but also, we need to reveal their properties that are essential in understanding the way they act. One of these properties is mechanism of action (MoA). The first step to study MoA is to determine minimal inhibition concentration according to EUCAST standards.[1] If the results are auspicious we can focus on specification of possible MoA. Currently, 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 antimicrobial 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 (inhibition of all mentioned biosynthetic pathways).[2]

This approach will be used in future to determine possible MoA of antimycobacterial compounds as well. The first step will be to find specific biomolecules typical for mycobacterial

biosynthetic pathways and their radioactively labelled equivalents respectively together with inhibitors of these pathways.

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

References

1. EUCAST DISCUSSION DOCUMENT E.DIS 5.1, DETERMINATION OF MINIMUM INHIBITORY CONCENTRATIONS (MICS) OF ANTIBACTERIAL AGENTS BY BROTH DILUTION. Clin. Microbiol. Infect. 9, 2003, 1-7.
2. NOWAKOWSKA, J., GRIESSER, H. J., TEXTOR, M., LANDMANN, R., KHANNA, N.: ANTIMICROBIAL PROPERTIES OF 8-HYDROXYSERRULAT-14-EN-19-OIC ACID FOR TREATMENT OF IMPLANT-ASSOCIATED INFECTIONS. Antimicrob. Agents Chemother., 57, 2013, 333–342.

Pharmacognosy and Toxicology of Natural Products

AMARYLLIDACEAE ALKALOIDS AS POTENT GSK 3B INHIBITORS

HULCOVÁ, D.,[1] BREITEROVÁ, K.,[1] HOŠŤÁLKOVÁ, A.,[1] ŠAFRATOVÁ, M.,[2] KUNEŠ, J.,[3] CAHLÍKOVÁ, L.[1]

[1] Department of Pharmaceutical Botany and Ecology, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

[2] Department of Pharmacognosy, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

[3] Department of Inorganic and Organic Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

e-mail: hulcovd@faf.cuni.cz

Alzheimer´s disease (AD) is one of the most frequent causes of dementia in the world. During AD in the brain occurs to pathological changes of some enzyme systems that result in loss of neurotransmitter acetylcholine and formation of amyloids plaques and neurofibrillary tangles (NFTs). As a results are brain damage, development of dementia and loos of cognitive functions. NFTs consisting of paired helical filaments, with the main component being hyperphosphorylated τ-protein. Phosphorylation of τ-proteins is primarily dependent on glycogen synthase kinase-3β (GSK-3β) and cyclin-dependent kinase 5.[1] Glycogen synthase kinase-3 is an ubiquitous serine/threonine kinase that plays a crucial role in numerous cellular functions, including cell cycle regulation, differentiation and proliferation, and gene expression by regulating a wide variety of substrates like glycogen synthase or tau-protein.[2]

In our ongoing study focused on Amaryllidaceae alkaloids, we have investigated thirty previously isolated alkaloids from Zephyanthes robusta, Nerine bowdenii, Chlidanthus fragrans and various Narcissus cultivars for their GSK-3β inhibition potential. For all compounds was measured percentage inhibition at concentration 50 μM. Inhibitory activity IC50 was determined for three compounds (masonine: 28.25 μM ± 4.05, 9-O-demethylhomolycorenine: 27.20 μM ± 10.80 and caranine: 31.54 μM ± 1.26). Since galanthamine is used in therapy of AD, Amaryllidaceae alkaloids are still promising goal in searching for new bioactive compounds.

References:

1. HAMANN, M., ALONSO, D., MARTÍN-APARICIO, E., FUERTES, A., PERÉZ-PUERTO, MJ., CASTRO, A., MORALES, S., NAVARRO, ML., DEL MONTE-MILLÁN, M., MEDINA, M.,

PENNAKA, H., BALAIAH, A., PENG, J., COOK, J., WAHYUONO, S., MARTÍNEZ, A.: Journal of Natural Products, 70, 2007, 1397-1405.

2. PLATTNER, F., ANGELO, M., GIESE, KP.: Journal of Biological Chemistry, 281, 2006, 25457-25465.

PHYTOCHEMICAL STUDY OF AMARYLLIDACEAE ALKALOIDS FROM *NARCISSUS CV.* CARLTON AND THEIR BIOLOGICAL ACTIVITY

AL MAMUN, A.,[1] CAHLÍKOVÁ, L.[1]

[1]ADINACO Reasearch Group, Department of Pharmaceutical Botany, Faculty of Pharmacy, Charles University, Hradec Králové, Czech Republic

Plants of Amaryllidaceae family are crucial in natural product research for its structurally diverse group of compounds as long as broad range of therapeutic potentiality. The most earliest evidence has been found at fourth century B.C.E. when Hippocrates of Cos used *Narcissus poeticus* L. oil of extract for the treatment of uterine tumors[1]. *Narcissus* cv. Carlton is ornamental plant under Amaryllidaceae family. Until now more than 600 Amaryllidaceae alkaloids signifying 18 skeletal types of alkaloids have been isolated, which comprise galanthamine, lycorine, haemanthamine, pancratistatine, pretazzetine, montanine, narciclassine and others. These active metabolites showed widespread therapeutic application as antitumor, antibacterial, antifungal, antimalarial, antiviral, cytotoxic, analgesic, acetylcholinesterase and butyrylcholinesterase inhibitors[2]. Among these active metabolites, galanthamine is most effective alkaloid and is clinically used for the treatment of Alzheimer's diseases (AD), since 2000. After this, searching of new alkaloids from Amaryllidaceae family have been accelerated. Haemathamine and haemanthidine are other important crinine type amaryllidaceae alkaloids demonstrated interesting anticancer activity against p53-mutated Caco-2 and HT-29 colorectal adenocarcinoma cells and human leukemic Jurkat cells[3], and moderate anti-influenza virus activity against N5H1 cell lines[4]. GC/MS analysis of *Narcissus* cv. Carlton showed interesting amount of compounds with typical spectra of Amaryllidaceae alkaloids. Some of them have been identified based on their mass-spectra. Unidentified peaks are assumed to be new structures of Amaryllidaceae alkaloids. So far, concentrated alkaloidal extract from 50kg of fresh bulb of *Narcissus* cv. Carlton has been prepared and will be separated by column chromatography to isolate Amryllidaceae alkaloids in pure form for biological tests and preparations of their derivatives.

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

References:

1) KORNIENKO, A., Evidente, A.: Chem. Rev., 108, 2008, 1982-2014.
2) JIN, Z.: Nat. Prod. Rep., 30, 2013, 849-869.
3) HAVELEK, R., SEIFRTOVA, M., KRALOVEC, K., BRUCKOVA, L., CAHLIKOVA, L., DALECKA, M., VAVROVA, J., REZACOVA, M., OPLETAL, L., BILKOVA, Z.: Phytomedicine, 21, 2014, 479–490.
4) MAOMAO, H., CHUNRONG, Q., OUDE, G., XIANMING, H., XUECHUAN, H.: RSC Adv., 5, 2015, 16562–16574.

USE OF HPCCC FOR ISOLATING ASTAXANTHIN ESTERS FROM MICROALGAE *HAEMATOCOCCUS PLUVIALIS*

FÁBRYOVÁ, T.,[1, 2] CHEEL, J.,[2] TŮMOVÁ, L.,[1]

[1] Department of Pharmacognosy, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

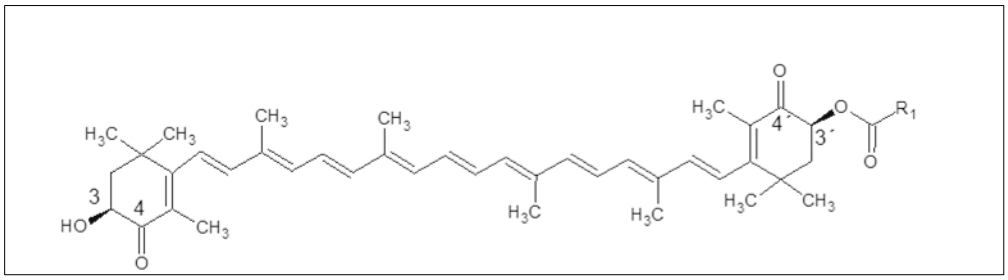
[2] Laboratory of Algal Biotechnology-Centre ALGATECH, Institute of Microbiology of the Czech Academy of Sciences, Czech Republic

e-mail: fabryovt@faf.cuni.cz

Astaxanthin (AXT), the most powerful antioxidant found in nature, is a red pigment with noticeable and varied bio-functional properties of great significance in human health and nutrition. The microalgae *Haematococcus pluvialis* is the major AXT natural source, in which this compound occurs mainly in the form of monoesters (AXTme) and diesters (AXTde). These esters have been shown to exert better benefits than the non-esterified AXT.[1,2] Given that there is an increasing demand for AXT from natural origin in the cosmetic, food and pharmaceutical sectors; therefore, the development of an efficient method for isolating AXT esters from microalgae is necessary. For facing this challenge, a high performance countercurrent chromatography (HPCCC) method will be developed, optimized and applied to obtain individual AXTme and AXTde from the microalgae *Haematococcus pluvialis*. HPCCC is a liquid–liquid chromatography technique that uses a liquid stationary phase without any solid support. Two immiscible liquid phases are used for the separation of target compounds. One of the two liquid phases (the stationary phase) is retained in the column by centrifugal force, whereas the other (the mobile phase) is pumped through the column.[3] In HPCCC, the chromatographic separation is based on the difference in the partitioning of each target compound between these two immiscible phases. Given that HPCCC uses a liquid stationary phase without a solid support, the method offers many advantages over traditional solid–liquid chromatography, such as the absence of irreversible adsorption of target molecules, high sample loading capacity and recovery, low risk of sample denaturation, and low solvent consumption.[4] This chromatographic method is considered a cost-effective, high-throughput and scalable technology for the extraction of bioactive substances from natural sources. In the present proposal, the chemical identity of the isolated AXTme will be determined by high-performance liquid chromatography–atmospheric pressure chemical ionization–tandem mass spectrometry

(HPLC–APCI–MS/MS). This work will provide a new chromatographic approach for the isolation of astaxanthins from microalgal biomass.

Fig 1. Representative chemical structure of AXTme.



The study was supported by SVV 260294 and Charles University Grant´s Agency (GAUK 1134217). The results of this investigation will be under patent application.

References

1. LORENZ R. T., CYSEWSKI G.R.: Trends Biotechnol., 18, 2000, 160–167.
2. RAO A. R., SINDHUJA H. N., DHARMESH S. M., SANKAR K. U., SARADA R., RAVISHANKAR G. A.: J. Agric. Food Chem., 61, 2013, 3842–3851.
3. CHEEL J., URAJOVÁ P., HÁJEK J., HROUZEK P., KUZMA M., BOUJU E., FAURE K., KOPECKÝ J.: Anal. Bioanal. Chem., 409, 2017, 917–930.
4. MICHEL T., DESTANDAU E., ELFAKIR C.: Anal. Bioanal. Chem., 406, 2014, 957–969.

ISOLATION OF AMARYLLIDACEAE ALKALOIDS FROM *ZEPHYRANTHES CITRINA*

KOHELOVÁ E.,[1] KUNEŠ, J.,[2] CAHLÍKOVÁ, L.[1]

[1]Department of Pharmaceutical Botany, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

[2]Department of Organic and Bioorganic Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

e-mail: kohelove@faf.cuni.cz

Zephyranthes is a genus of bulbous perennials belonging to family Amaryllidaceae. The plants of this family are used by native peoples of different countries for treating various diseases. The genus *Zephyranthes* is one amongst 75 genera under this family. It consists of about 90 species and out of which few 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 have been reported in *Zephyranthes citrina* (lycorine, lycorenine, galanthine, haemanthamine, oxomaritidine, maritidine, haemanthidine, vittatine, galanthine, narcissidine).[1]

The summary ethanolic extract was prepared from the fresh bulbs of *Zephyranthes citrina*. More than six hundred fractions were collected by column chromatography (on Al_2O_3). Fractions were pooled into subfractions. So far, two pure alkaloids have been isolated. The isolated compounds were identified as haemanthamine and galanthine by comparison with the literature data and results of MS and NMR studies.

The study was supported by SVV 260 412.

REFERENCES:

1 KATOCH, D.; SINGH, B. Phytochemistry and pharmacology of genus *Zephyranthes*. Med Aromat Plants, 2015, 4.212: 2167-0412.1000.

NMR ELUCIDATION OF ALKALOIDS ISOLATED FROM *Magnolia* × *Soulangeana*

MAŘÍKOVÁ, J.,[1] HOŠŤÁLKOVÁ, A.,[2] KUNEŠ, J.,[1]

[1] Department of Organic and Bioorganic Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

[2] Department of Pharmaceutical Botany, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

e-mail: marikoj2@faf.cuni.cz

Eight alkaloids were obtained from *Magnolia* × *soulangeana* Soul.-Bod at the Department of Pharmaceutical Botany, Faculty of Pharmacy, Hradec Králové. The structure of two of them was predicted by MS analysis and confirmed by NMR analysis – liriogenine and *N*-methylcoclaurine. Six of isolated substances had to be characterized employing basic ^{1}H and ^{13}C NMR 1D experiments and advanced 2D experiments as gHMBC, gHSQC, gCOSY and NOESY. The identified structures were compared with available literature. All of these isolated compounds have been already described in the literature as *N*-norarmepavine, armepavine, β-carboline, asimilobine, coclaurine, magnolamine.

This work was supported by Czech Science Foundation (project GA ČR 15-07332S) and Charles University (project SVV 260 401).

PHYTOCHEMICAL ANALYSIS AND BIOLOGICAL ACTIVITY OF SELECTED MEDICINAL PLANTS WITH A FOCUS ON TESTING THE IMMUNOMODULATORY AND TYROSINASE ACTIVITIES

VRABEC, R., TUMOVA, L.

Department of Pharmacognosy, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

e-mail: vrabecr@faf.cuni.cz, tumova@faf.cuni.cz

Given the early stage of research, this study does not provide any valid data yet. The presentation will be focused on the introduction of selected plants and planed methods.

The first selected plant is *Scutelaria baicalensis* L. (Baical skullcap, family *Lamiaceae*) - one of the medicinal herbs with a long history of usage in traditional Chinese medicine. Researches over the last decades revealed and confirmed a variety of important properties of content substances (over 40 flavonoids, etc.), which contributed to the general awareness of this herb. *Scutellaria baicalensis* extracts are part of dieatary supplements. Given the worldwide prevalence of deaths caused by cardiovascular diseases and the increasing challenge of infections, immunomodulatory and antiaggregation activity must be demonstrated and elucidated. Attention will be also put on antiparasitic and antityrosinase activities, whether the plant exhibits these to this day untested effects.

The second selected medicinal plant for this study is *Azorella compacta* Phil. (syn. *A. yareta*, Llareta, family *Apiaceae*). It is a cushion shrub grown at altitudes of the Ands in South America's puna. Natives traditionally use the plant in a form of tea to treat a cold, pain, rheumatism, diabetes, and also as a stomachicum and diureticum. Experiments with aqueous extracts proved the antioxidant and immunomodulatory effect of contained polyphenols, but it is still unknown which specific substances are responsible for the effects. In addition to the isolation and evaluation of the biologically active substances, antiaggregatory, antityrosinase and antiparasitic activities will be tested as well.

COPPER CHELATION ACTIVITIES OF ISOLATED COMPOUNDS FROM *SILYBUM MARIANUM* - STRUCTURE–ACTIVITY RELATIONSHIP

KARLÍČKOVÁ, J.,[1] TVRDÝ, V.,[2] VALENTOVÁ, K. ,[3] KŘEN, V. ,[3] MLADĚNKA, P.[2]

[1] Department of Pharmaceutical Botany, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

[2] Department of Pharmacology and Toxicology, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

[3]Laboratory of Biotransformation, Institute of Microbiology, Academy of Sciences of the Czech Republic, Czech Republic

e-mail: karlickova@faf.cuni.cz

Silybum marianum (L.) Gaertn. is a medicinal herb from family Asteraceae. It is frequently used for it hepatoprotective properties in the form of a mixture known as silymarin. Silymarin is composed from flavonolignans including silybin A and B, isosilybin A and B, silychristin, isosilychristin and 2,3-dehydrosilybin and also flavanonol taxifolin. Pharmacokinetics of pure silybin diastereoisomers and identification of their metabolites in rat plasma was recently introduced.[1]

The aim of the study was to test four isolated compounds silybin A and B, silychristin and 2,3-dehydrosilybin from silymarin in order to assessment their copper chelation properties at (patho)physiologically relevant pH conditions by use of our previously published approaches.[2,3]

All tested compounds chelated cupric ions under mildly competitive conditions (hematoxylin method). 2,3-dehydrosilybin was shown as the most potent chelator in a more competitive assay (bathocuproin method) while other flavonolignans did not chelate neither cupric and cuprous ion in this experiment. Evaluation of cupric reduction demonstrated that all tested flavonolignans were potent reducing agents.

In conclusion, 2,3-dehydrosilybin had the strongest copper chelating properties while silychristin was the most potent copper reductant.

The study was supported by PRVOUK P40.

References

1. MARHOL, P., BEDNÁŘ, P., KOLÁŘOVÁ, P., VEČEŘA, R., ULRICHOVÁ, J., TESAŘOVÁ, E., VAVŘÍKOVÁ, E., KUZMA, M., KŘEN, V.: J. Funct. Foods, 14, 2015, 570–580.
2. KŘENEK, K., MARHOL, P., PEIKEROVÁ, Ž., KŘEN. V., BIEDERMANN, D.: Food Res. Int. 65, 2014, 115-120.
3. ŘÍHA, M., KARLÍČKOVÁ, J., FILIPSKÝ, T., MACÁKOVÁ, K., HRDINA, R., MLADĚNKA, P.: J. Inorg. Bioch., 123, 2013, 80–87.

ALKALOIDS OF *Vinca minor* L. AND THEIR EFFECT ON ACTIVITY OF SELECTED ENZYMES AS BENEFIT TO PROGRESS OF ALZHEIMER'S DISEASE

LOČÁREK, M.,[1] KUNEŠ, J.,[2] HRABINOVÁ, M.,[3] HOŠŤÁLKOVÁ, A.,[1] CHLEBEK, J.,[1] KUČEROVÁ, J.,[1] VÍTOVCOVÁ, A.,[1] OPLETAL, L.,[1]

[1] Department of Pharmaceutical Botany, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

[2] Department of Organic and Bioorganic Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

[3] Department of Toxicology and Military Pharmacy, Faculty of Military Health Sciences, University of Defence, Czech Republic

e-mail: locarekm@faf.cuni.cz

Vinca minor L. is an ornamental plant from Apocynaceae family commonly used in gardens, but it is also a source of alkaloids. So far, more than 45 alkaloids of indole type have been isolated from this plant. Indole type alkaloids are known as the source of potential anticholinesterase inhibitors for the treatment of Alzheimer's disease[1].

In our study 62 kg of dried aerial parts of *V. minor* were three times extracted with EtOH, the solvent was evaporated under reduced pressure, the extract was dissolved with hot water, and filtered. The aqueous solution was adjusted to pH=9–9.5 with 25% NH_4OH and alkaloids were five times extracted with $CHCl_3$. 454 g of crude extract was obtained after evaporation.

The mixture of the total alkaloids was divided by means of column chromatography into sixteen parts containing alkaloids. Chromatography was performed on alumina using gradually enriched petrol–chloroform and chloroform–ethanol mixtures for elution. Subsequently combination of flash and repeated thin-layer chromatography led to the isolation of pure compounds.

So far, more than 8 alkaloids were isolated in our study. Chemical structures of compounds were elucidated by optical rotation, spectroscopic and spectrometric analysis (NMR, MS) and comparison with literature data. Next the human blood acetylcholinesterase (HuAChE) and human serum butyrylcholinesterase (HuBuChE) inhibitory activities of them were studied. In addition selected compounds were tested for recombinant prolyl oligopeptidase (POP)

inhibitory activity and in some cases the parallel artificial membrane permeability assay (PAMPA) was performed.

References

1. KONRATH, E. L., PASSOS, C. dos S., KLEIN-Junior, L. C., HENRIQUES, A, T.: Journal of Pharmacy and Pharmacology, 65(12), 2013, 1701–1725.

Pharmaceutical Technology

MONOLAYER STUDY OF GLUCOSYLCERAMIDE-TO-CERAMIDE PROCESSING DURING SKIN BARRIER FORMATION

NOVÁČKOVÁ, A.,[1] ŠVECOVÁ, B.,[1] VÁVROVÁ, K.,[2] ZBYTOVSKÁ, J.[1]

[1] Department of Pharmacutical Technology, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic;

[2] Department of Organic And Bioorganic Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic;

e-mail: novacka1@faf.cuni.cz

Ceramides (Cer) together with free fatty acids and cholesterol form the intercellular space of the uppermost skin layer, *stratum corneum* (SC). This lipid matrix represents the proper skin barrier, which protects mammalian organisms against environmental factors (endogenous substances, physical radiation) and prevents body from water loss. All Cer subtypes are synthesized from their polar precursor glucosylCer (GlcCer) by removing the polar part by hydrolytic enzyme β-glucocerebrosidase (GlcCer-ase). A lack of this enzyme leads to accumulation of precursors and a disturbed skin barrier function, e.g. in type 2 Gaucher disease.

The goal of this work was to study the processing of GlcCer to Cer by monolayer lipid models of SC. The control monolayers contained GlcCer, free fatty acids and cholesterol in equimolar ratio. In order to study the process of Cer formation, GlcCer were gradually (75, 50, 25, 10, 5 %) replaced by Cer. As a liquid subphase under the monolayers, we tested phosphate (pH 7.4) and acetate (pH 5.0) buffer. At low surface pressure (1.5 $mN.m^{-1}$), the lipids organize more spontaneously at pH 5.0 than at pH 7.4, apart from the mixtures with 25 and 50 % of GlcCer, which are not influenced by pH. With increasing surface pressure (20 $mN.m^{-1}$) there is similar trend at pH 7.4 like at lower pressure, however, at pH 5.0 the molecular area of the mixtures is lower compared to pH 7.4. Surprisingly low area per molecule at 20 $mN.m^{-1}$ (tighter organization of lipids) of mixture with 50 % GlcCer corresponds with low permeability of multilayer model membranes. At pH 5.0, compressibility is lower in mixtures containing GlcCer than without GlcCer. By contrast, at pH 7.4, compressibility of mixtures containing GlcCer is higher than without it. Langmuir isotherms showed that the dependence of precursor concentration in monolayers on tight arrangement of lipid molecules is nonlinear and that pH

slightly affects formation of monolayers. It seems that the structure of the polar head influences the mutual interactions between lipids during the formation of the SC lipid membranes.

The study was supported by the Czech Science Foundation (16-25687J) and the Charles University in Prague (GAUK 184217 and SVV 260 401).

SILK FIBROIN AND ITS APPLICATION IN PHARMACEUTICAL TECHNOLOGY

KOŽÁK J.,[1,2] RABIŠKOVÁ M.,[1] LAMPRECHT A.[2]

[1] Department of Pharmaceutical Technology, Faculty of Pharmacy in Hradec Králové, Charles University in Prague, Czech Republic

[2] Department of Pharmaceutical Technology and Biopharmaceutics, Institute of Pharmacy, University of Bonn, Bonn, Germany.

e-mail: kozakja1@faf.cuni.cz

Silk fibers produced by silkworm *Bombyx Mori* are used in medicine for centuries as biocompatible and biodegradable surgical sutures. The native silk fibers after undergoing a process called degumming, during which water soluble protein sericin is removed, are composed of protein polymer called silk fibroin (SF).[1] Being naturally produced polymer, its renewable and biodegradable features make SF an attractive environmentally friendly polymer.

So far, the main applications of SF drug delivery systems have been focused in tissue engineering. SF in crystalline form is insoluble in water and any common organic solvent.[1] These properties make the polymer suitable for sustained release drug delivery applications.

In our work we produced sustained release matrix tablets. Due to the inherent insolubility of SF in ethanol, these tablets are resistant to so called "alcohol-induced dose dumping". A phenomenon which leads to faster or immediate release of all drug content, when co-administered with ethanolic beverages and potential overdosing. [2,3] The issue is especially relevant in case of opioid analgetics due to the well-known pharmacodynamic interaction and potentifying effect of ethanol on central nervous system.[3] Therefore oxycodone hydrochloride and tramadol hydrochloride have been selected as model compounds. The results show slower drug release with increasing ethanol concentration in dissolution medium and different swelling behavior.

The study was supported by SVV 260 183

References

1. WENK E. et.al., J. Control. Release. 2011 Mar 10;150(2):128-41.

2. JEDINGER N., et.al. Eur. J. Pharm. Biopharm. 2014 Jul 31;87(2):217-26.

3. TRAYNOR M.J., et.al. Drug Dev. Ind. Pharm. 2008 Jan 1;34(8):885-9.

POLYMERIC NANOPARTICLES FOR INTRACELLULAR DELIVERY

KUBAČKOVÁ, J., HOLAS, O., DITTRICH, M.

Department of Pharmaceutical Technology, Faculty of Pharmacy in Hradec Králové, Charles University, Czech republic

e-mail: kubackja@faf.cuni.cz

Multiple pathologies result in liver inflammation[1]. Inflammatory response is a complex process and intracellular receptors[23] in macrophages have been found to regulate it. Appropriate carries, carrying ligands to these receptors, at size ranging from 100 to 300 nm and negative surface charge can be passively targeted to liver and uptaken by macrophages[4]. Subcellular dimensions of nanocarriers enable their uptake by targeted cells and action on intracellular level. Poly(DL-lactic-co-glycolic acid) (PLGA) is a biocompatible polymer, its biodegradability results in a sustained release profile of the encapsulated drug[5]. In our study, both linear and branched PLGA polymers were utilised. Screening through various types of stabilizers, in combination with different preparation methods was carried out. Nanoprecipitation method was used to encapsulate a fluorescent dye Rhodamine B also known as a ligand for aforementioned intracellular receptors. Particle size, polydispersity index and zeta potential confirmed stable negatively charged nanoparticles sized from 180 to 320 nm with polydispersity of acceptable values. Dissolution tests indicate desired sustained release profiles in range of days varying in respect to the utilized polymer. Such NP parameters together with promising relative encapsulation effiency up 90% meet the requirements for targeted drug delivery formulations.

The study was supported by SVV 260 401 and GACR 303/12/G163.

References

1. BARTNECK, M., WARZECHA, K. T., TACKE, F.: Hepatobiliary Surg. and Nutr., 3, 2014, 364–376.
2. PAVEK, P., HYRSOVA, L., SMUTNY, T., CARAZO, A., MORAVCIK, S., MANDIKOVA, J., GERBAL-CHALOIN, S.: Br. J. Pharmacol., 173, 2016, 1703–1715.
3. ZHANG, X.-Q., EVEN-OR, O., XU, X., VAN ROSMALEN, M., LIM, L., GADDE, S., FISHER, E. A.: J. Control. Release, 4, 2015, 228–236.
4. TAMMAM, S. N., AZZAZY, H. M. E., LAMPRECHT, A.: J. Biomed. Nanotechnol., 11, 2015, 555–577.
5. SOPPIMATH, K. S., AMINABHAVI, T. M., KULKARNI, A. R., RUDZINSKI, W. E.: J. Control. Release, 70, 2001, 1-20.

STARLIKE POLYMERIC NANOPARTICULATE DRUG CARRIERS

MARTISKA, J.,[1] SNEJDROVA, E.,[1] DRASTIK, M.,[1] DITTRICH, M.[1]

[1] Department of Pharmaceutical technology, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

e-mail: martisju@faf.cuni.cz

Aim of our work is to prepare polymeric nanoparticles from originally synthesized biodegradable branched polyesters with intent of achieve prolonged response, and to reduce adverse effects of selected incorporated drugs (terbinafine hydrochloride, rifampicin, siRNA). We used aliphatic starlike polyesters branched on tripentaerythritol of average molecular weight (Mw) 17400, and branched on poly(acrylic acid) with Mw 14400. Nanoparticles were prepared using modified nanoprecipitation method.[1] Firstly, we prepared organic phase consisted from solution of polyester in dimethyl sulfoxide, in which specific amount of drug was dissolved. Different concentration of surfactants in water represented aquaeous phase. The aqueous phase at room temperature was placed on magnetic stirrer and stirred at medium speed. Then the organic phase was added dropwise into the aqueous phase, stirring proceeded for 30 minutes. We monitored multiple parameters such as particles size, polydispersity and zeta potential using Zetasizer Nano ZS by Malvern. The size of the nanoparticles were 100-250 nm, and could be modify by concentration of the polyester and mixing technique of phases. For analysis, we used HPLC, fluorometer, or spectrophotometer, according to 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] Examined polyesters are perspective, original, and suitable for further observation.

The study was supported by SVV 260 401

References

1. FESSI, H., F. PUISIEUX, J.PH. DEVISSAGUET, AMMOURY, N., BENITA, S.: Int. J. Pharm., 55, 1989, R1-R4.
2. DITTRICH, M., SNEJDROVA, E.: J. Pharm. Sci., 103, 2014, 3560-3566

THE COMPRESSIBILITY STUDY OF PELLETS, POWDER MICROCRYSTALLINE CELLULOSE AND THEIR MIXTURES

TRPĚLKOVÁ, Ž., ONDREJČEK, P., ŠKLUBALOVÁ, Z.

Department of Pharmaceutical Technology, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

e-mail: trpelkoz@faf.cuni.cz

Pellets are very popular in pharmaceutical technology because they have spherical shape, smooth surface, narrow particle size distribution and excellent flow properties. They are very often used as drug carriers; a drug is either included into a pellet matrix or onto a pellet surface. However, according to our previous experiments, pellets themselves are not suitable to be used as a tablet filler and they need to be mixed with other additives to achieve a mixture with appropriate compaction properties.[1]

Hence, this study deals with the evaluation of compressibility of microcrystalline cellulose pellets Cellets® 100 (C100), powder microcrystalline cellulose Comprecel 102 (MCC) and their mixtures. Nine mixtures with different ratio of C100 and MCC were prepared and compacted under ten compaction forces in a range of 2 – 20 kN. The radial strength[2] and elasticity of prepared tablets were evaluated.[3,4]

The results showed that the radial strength of the tablets increased with the rising concentration of microcrystalline cellulose in the mixture. In accordance with the generally recommended optimal range of the tablet tensile strength 0,56 - 1,12 MPa, the mixtures containing 60 and 70 % of pellets were the most promising for the tablet preparation. However, further studies involving tablet friability, disintegration and dissolution testing are necessary.

The study was supported by SVV 260 401.

References

1. TRPĚLKOVÁ, Ž.: Diplomová práce, Univerzita Karlova, Farm. Fakulta v Hradci Králové, 2016
2. FELL, J. T.; NEWTON, J. M.: J. Pharm. Sci., 59, 1970, 688-691.
3. PICKER-FREYER, K. M.; BRINK, D.: AAPS PharmSciTech, 2006, 7(3), 152-161.
4. MAGANTI, L.; ÇELIK, M.: Int. J. Pharm., 1993, 95(1-3), 29-42.

PRE-FORMULATION STUDIES OF LIQUISOLID SYSTEMS

VRANÍKOVÁ, B., SVAČINOVÁ, P., ŠKLUBALOVÁ, Z.,

Department of Pharmaceutical technology, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

e-mail: vranikovab@faf.cuni.cz

One of the trends of modern pharmaceutical technology is a formulation of dosage forms with enhanced dissolution rate and improved bioavailability of poorly soluble active ingredients. The scientific literature describes different methods improving bioavailability, however, the preparation of liquisolid systems (LSS) seems to be one of the most promising and innovative techniques among them. The basic principle of LSS formulation is a conversion of the drug in the liquid state into an apparently dry powder by its blending with specific carriers. The carrier particles are subsequently coated with a highly absorptive material giving the LSS the desirable flow and compression characteristics.[1]

Properties of LSS can be influenced by many factors, such as a solubility of the drug in the chosen solvent, properties of used excipients and calculations of the required amounts of carrier and coating material. Therefore, the presented work aimed at the determination of the optimal combination of the solvent, carrier and coating material for the preparation of LSS. The flowable liquid retention potential of several carriers and coating materials for three non-volatile hydrophilic solvents was measured. According to the obtained results, the compressible liquid retention potential of the granulated form of magnesium aluminometasilicate (Neusilin® US2) for these solvents was evaluated. It was observed that the tablets containing 55 % of polyethylene glycol 400 and 60 % of polyethylene glycol 200 fulfilled all requirements given by the Ph.Eur.. Subsequently, the processing properties of these mixtures, e.g. the optimal compression pressure and compaction behavior with the varying amounts and types of coating materials etc., were investigated for the possible use in dosage forms.

The study was supported by Progres Q42 Výzkum a studium léčiv.

References:

1. VRANÍKOVÁ, B., GAJDZIOK, J.: Acta Pharmaceutica, 63, 2013, 447-465.

Pharmaceutical Analysis and Bioanalytical chemistry

UHPLC-MS/MS METHOD FOR MONITORING OF ARGININE METABOLISM IN CHRONIC WOUNDS

JAVORSKÁ, L. [1,2], KUJOVSKÁ KRČMOVÁ, L. [1,2], HAZUKOVÁ, N. [1], SOBOTKA, L. [1,3], SOLICH, P [1].

[1] Department of Analytical Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

[2] Department of Clinical Biochemistry and Diagnostics, University Hospital Hradec Králové, Czech Republic

[3] 3rd Internal Gerontometabolic Clinic, University Hospital Hradec Králové, Czech Republic

e-mail: javorskl@faf.cuni.cz

Wound healing presents very complex and dynamic process. Metabolism of amino acid arginine plays an important role in this process. It is a precursor for synthesis of nitric oxide, proline, hydroxyproline and polyamines. Arginine also stimulates the release of growth hormone, insulin-like growth factor 1 and other compounds that significantly influence wound healing[1].

The aim of this study was developed UHPLC-MS/MS method for the determination of L-arginine and its metabolites L-ornithine, L-citrulline, agmatine in a fluid obtained from non healing wounds. Several stationary phases were tested and compared for the optimal retention and separation of these polar analytes: HILIC, F5, two modifications of C18 and BEH Amide. The optimal conditions were applied for the determination of these molecules in real patient samples with simple sample pretreatment procedure. The study was performed using Nexera® UHPLC system with a Triple Quadrupole Mass Spectrometer LCMS 8030 (Shimadzu, Japan) operating in ESI positive mode.

In future, this method will be useful for the direct monitoring of arginine metabolism in chronic wound which can contribute to improve the therapy.

The study was supported by project SVV 260 412, MH CZ – DRO (UHHK, 00179906).

References

1. STECHMILLER JK, CHILDRESS, B, COWAN L.: Nutr Clin Pract, 20, 2005, 52 – 61.

DEVELOPMENT OF UHPLC METHOD FOR STABILITY STUDY OF OMEPRAZOLE SUSPENSIONS

KUČEROVÁ, K.,[1,2] REISKÁ, V.,[1] KUJOVSKÁ KRČMOVÁ, L.,[1,2] MATYSOVÁ, L.,[1]

[1] Department of Analytical Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

[2] Department of Clinical Biochemistry and Diagnostics, University Hospital in Hradec Králové, Czech Republic

e-mail: kucerok1@faf.cuni.cz

Omeprazole is one of the most widely used drugs from the group of proton-pump inhibitors (PPIs). Due to the instability of omeprazole in acidic conditions, enteric-coated tablets or extended-release capsules are commonly used dosage forms. This kind of drug administration is not convenient in pediatric and another patients having problems to swallow solid dosage form. Thus, individually prepared oral suspensions of omeprazole facilitate the administration and the dosage.[1,2]

A simple and fast ultra-high-performance liquid chromatography method with UV detection for the separation and quantification of omeprazole and its impurity of omeprazole and methylparaben (the internal standard) in six extemporaneous suspensions was developed and fully validated. Separation was performed using 1.7 μm porous shell particles (Kinetex^{TM} C18, 50 × 2.1 mm) in combination with an isocratic elution of a phosphate buffer and acetonitrile. The separation of all compounds was achieved within 2 minutes. The method was successfully applied during a stability evaluation of the developed formulations, which are now being used in the therapy of acid-related disorders in paediatric patients. Results of stability study will be presented.

The study was supported by Project SVV 260 412.

References

1. BURNETT, J., BALKIN, E.: Am. J. Health Syst. Pharm., 63, 2006, 2240–2247.
2. ISRAEL, D., HASSALL, E.: J. Pediatr. Gastroenterol. Nutr.,27, 1998, 568–579.

CHROMATOGRAPHIC METHOD FOR THE ASSESSMENT OF VITAMIN B_1 AND B_6 DERIVATIVES IN WHOLE BLOOD

JENČO, J., [1, 2] KUJOVSKÁ KRČMOVÁ, L., [1, 2] SOLICHOVÁ, D., [2] SOLICH, P., [1]

[1] Department of Analytical chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

[2] Department of Clinical Biochemistry and Diagnostics, University Hospital in Hradec Králové, Czech Republic

e-mail: jencoj@faf.cuni.cz

Derivatives of thiamine and pyridoxine have crucial role in cellular metabolism. Discrepancies in their status have serious deleterious effects. Manifestation is often vague and may be easily overlooked [1]. Therefore, monitoring of vitamin status has large importance especially in patients with intensive care. Methods for simultaneous determination of thiamine, its mono- and diphosphate derivatives with an active form of vitamin B_6 - pyridoxal-5-phosphate are still not widely established in diagnostics [2].

Novel HPLC-FLD method with pre-column derivatization was developed, optimized and validated for the simultaneous analysis of thiamine and its derivatives with pyridoxal-5-phosphate in whole blood. Separation was accomplished by Meteoric Core-BIO C-18 core-shell column (100 × 4.6 mm, YMC, Germany) protected with SecurityGuard C18-WP guard column (10 × 4.6 mm, Phenomenex, USA). During gradient elution all target compounds were eluted within 15 minutes. Limits of detection are below clinically important values. Recoveries were in the range of 90 to 110% for all analytes. Bioanalytical method will be further implemented into routine practice and used primarily for the determination of thiamine and its derivatives in patients with supplementary nutrition therapy, where fluctuating level of metabolically active vitamins are associated with the occurrence of possible complications.

The study was supported by Project SVV 260 412 and by University Hospital in Hradec Králové, 00179906.

References

1. JENČO, J., KUJOVSKÁ KRČMOVÁ, L., SOLICHOVÁ, D., SOLICH, P.: J. Chromatogr. A, 1510, 2017, 1-12.
2. COLLIE, J.T.B., GREAVES, R.F., JONES, O.A.H., LAM, Q., EASTWOOD, G.M., BELLOMO, R.: Clin. Chem. Lab. Med., 55(11), 2017, 1652–1668.

MIXED-MODE STATIONARY PHASES: EFFECT OF MOBILE PHASE TYPE AND ITS IONIC STRENGTH ON SEPARATION

CHRENKOVÁ, L.,[1] JAKUBEC P.,[1] SCHOEBE, S.,[2] DOUŠA, M.,[3] NOVÁKOVÁ, L.,[1]

[1] Department of Analytical Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

[2] Faculty of Chemistry and Earth Sciences, Friedrich Schiller University Jena, Germany

[3] Zentiva, k.s. Praha, U Kabelovny 130, 102 37 Praha 10, Czech Republic

e-mail: chrenklu@faf.cuni.cz

The analysis of weak protonated bases by RP-HPLC has always been challenging when using acidic or neutral mobile phases due to significant peak tailing and subsequent decrease of chromatographic resolution and increase of detection limits. Charged Surface Hybrid (CSH) columns are mixed mode stationary phases based on silica/ethylene hybrid particles (BEH). They are prepared by binding a controlled number of amine groups to these particles. Therefore, higher peak capacities for separations of positively charged analytes when using the low ionic strength acidic mobile phases, as well as symmetric peak shapes, and higher efficiency are possible due to positively charged pore surface with protonated amine groups at pH less than 3.

The screening was performed on three CSH columns (CSH C18, CSH Phenyl-Hexyl, CSH Fluoro-Phenyl) using gradient elution and pharmaceutically important compounds with basic, neutral, and also acidic properties. The study was carried out on UHPLC system Acquity UPLC with UV detection at 254 nm. The mobile phase consisted of acetonitrile and acidic aqueous solution or buffer with low ionic strength. The analyses with aqueous solutions (containing formic acid, hydrochloric acid, sulfuric acid, phosphoric acid, methanesulfonic acid, or perchloric acid) and buffers with different pH (1.0 – 3.1) and concentrations (0.01 – 0.5 %) were carried out.

Thus, monovalent and multivalent mobile phases with different ionic strengths were tested. The ionic strength was the decisive parameter for retention time shifts of strongly basic compounds and was correlated as the logarithm of mobile phase ionic strength.

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 260 412/2017

ENRICHING OF PREPARED FLAT SHEET POLYSULFONE MEMBRANE FOR SEPARATION OF BIOMOLECULES WITH ANTIOXIDANT A-TOCOPHEROL AND A-LIPOIC ACID

KOHLOVÁ, M.,[1,2] AMORIM, C.,[2] ARAÚJO, A.,[2] SOLICH, P.,[1] MONTENEGRO, C.,[2]

[1] Department of Analytical Chem., Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

[2] REQUIMTE/LAQV Department of Applied Chemistry, Faculty of Pharmacy, University of Porto, Portugal

e-mail: kohlm5aa@faf.cuni.cz

Patients suffering by chronic kidney disease, undergoing frequent haemodialysis (HD) treatment present significantly elevated level of oxidative stress (OS) and chronic inflammation caused by HD treatment itself, besides the disease conditions. The long-term contact of blood with artificial materials causes overstimulation of polymorphonuclear cell with subsequent inflammatory reaction and elevated OS leading to other severe complications by these patients. Polysulfone (PS) is nowadays the most used polymer for HD membranes due to its improved biocompatibility. Nevertheless, to minimize the negative effect of HD procedure, the bioactive hollow-fibre PS membranes modified with vitamin E are commercially used. [1]

In the present work, flat sheet PS membrane was prepared, for laboratory purposes to mimic HD procedure, using spin coating technique, followed by phase inversion process. Developed PS membrane was optimized and tested to fulfil the removal characteristics required for HD. The antioxidant-enriched membranes were prepared by dissolving α-tocopherol or α-lipoic acid in N-methyl-2-pyrrolidon, during PS solubilisation process. The release of α-tocopherol or α-lipoic acid from the membrane, during the phase inversion, was quantified by fluorometry and UV spectrophotometry, respectively. Both types of enriched membranes were tested for separation characteristics and their antioxidant activity was evaluated by FRAP assay.

Obtained results show that membranes enriched with α-lipoic acid, compared to α-tocopherol, show better separation characteristics of biomolecules. However, the antioxidant activity was higher by the membranes coated with α-tocopherol.

To assess the capability of both types of enriched membranes to reduce OS, the in vitro tests with blood from HD patients should be further conducted.

This work was supported by the Charles University Grant Agency, project GAUK No. 860216

References

1. WENTEN, I.G., et al.: Journal of Membrane Science and Research, 2(2), 2016, 78-89.

APPLICABILITY OF ULTRA-HIGH PERFORMANCE SUPERCRITICAL FLUID CHROMATOGRAPHY IN PHARMACEUTICAL QUALITY CONTROL

PLACHKÁ K., NOVÁKOVÁ, L.

Department of Analytical Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

e-mail: plachkka@faf.cuni.cz

The interest in ultra-high performance supercritical fluid chromatography (UHPSFC) separations in the field of impurity profiling is continuously growing. About seventy different pharmaceuticals were analyzed by UHPSFC with UV and mass spectrometry detection in first part of this study. Ten different quality control mixtures were selected and on a top of this, several beta blockers were added to this study in order to reflect the behavior of very basic compounds. Eight stationary phases (Torus diol, Torus diethylamine, Torus 2-picolylamine, Torus 1-amino anthracene, BEH 2-ethylpyridine, BEH, CSH pentafluorophenyl and HSS C18 SB), 3 modifiers (methanol, ethanol, isopropanol), 3 modifier blends (methanol/acetonitrile, methanol/ethanol, ethanol/acetonitrile), and 5 additives in methanol (0.1% formic acid, 10 mmol/L ammonium formate, 10 mmol/L ammonium acetate, 0.1% ammonium hydroxide and 2% water) were tested in an attempt to find the most generic UHPSFC conditions. This approach resulted in UHPSFC methods for the determination of composition of 10 quality control mixtures. Their validation was necessary to prove their applicability in pharmaceutical quality control and for the determination of impurities at low concentrations. Using the best chromatographic conditions that emerged from the screening, four mixtures (atomoxetin, atorvastatin, estradiol, ticagrelor) were almost baseline separated. However, they required optimization, mostly due to limited resolution between active pharmaceutical ingredient and following impurity. Six mixtures (abiraterone, ezetimibe, enzatulamid, agomelatine, vardenafil, dasatinib) were separated completely and generic methods used in the screening were validated either directly or after a fine tuning. The selected validation parameters followed the ICH guideline and included system suitability test, linearity, accuracy, and precision.

The study was supported by the Project of Specific Research, SVV No. 260412/2017, and by the STARSS project (Reg. No. CZ.02.1.01/0.0/0.0/15_003/0000465) co-funded by ERDF.

NANOFIBROUS POLYMERS AS EXTRACTION SORBENTS IN A SAMPLE PREPARATION

RAABOVÁ, H., HÁKOVÁ, M., CHOCHOLOUŠOVÁ HAVLÍKOVÁ, L., ŠATÍNSKÝ, D.

Department of Analytical Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

e-mail: raabovah@faf.cuni.cz

The nanofibers and their application are very popular topic of current research. Many papers about the nanofibrous polymers application in different fields of science have been published. One of this is an analytical chemistry, especially in a sample preparation procedure. This is an important part of the analysis when interferences are removed from a sample and an analyte is pre-concentrated. At this time, new trends in the sample preparation methods are focused on using lower sample volumes, to achieve a higher specifity and selectivity, a lower consumption of organic solvents and fully automated methods. One of these trends is a search for new sorbent materials for solid phase extraction (SPE). SPE is the most used sample preparation method for liquid samples because of its simplicity and wide range of application.

The nanofibers have a good potential to be one of new sorbents for SPE trough their high specific surface area. In this project, polyamide 6 nanofibers prepared by electrospinning at the Technical University of Liberec were tested. These nanofibers were packed into a SPE cartridge and their extraction efficiency was tested and evaluated for several pharmaceutical substances, namely parabens, steroids, flavonoids, and lipophilic insecticides fenoxycarb and permethrine. A selection of these substances was based on their different polarity, hydrophilic-lipophilic properties and molecular weight. The sample concentration and extraction conditions were optimized at the beginning of this experiment. HPLC coupled to spectrophotometric detection was utilized for the evaluation of the amount of extracted analytes.

Some practical aspect of using the nanofibers (e.g. fabrication, packing and time of extraction) were also studied in this work.

The authors would like to acknowledge financial support of specific research, no. SVV 260 412.

AUTOMATED CONTINUOUS IN-SYRINGE DISPERSIVE LIQUID-LIQUID EXTRACTION AND BACK-EXTRACTION FOR THE DETERMINATION OF NITROPHENOLS IN SURFACE WATER

FIKAROVÁ, K., HORSTKOTTE, B., SKLENÁŘOVÁ, H.

Department of Analytical Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

e-mail: fikaroka@faf.cuni.cz

"Continuous magnetic stirring-assisted dispersive liquid-liquid microextraction" followed by dispersive analyte back-extraction to an aqueous acceptor phase is presented as a novel automated sample pretreatment method for preconcentration of an analyte from a large volume of the liquid sample. The procedure was automated using Lab-In-Syringe (LIS) technique. Mono-nitrophenols (*o*-, *m*-, and *p*-) were selected as model analytes known for their environmental impact.

The LIS flow technique uses the void of the syringe pump of a Sequential Injection Analysis (SIA) system as a size-adaptable extraction chamber. A continuous flow of sample provided by an additional pump is enabled by the secondary inlet in the syringe. First, the extraction solvent was aspirated into the syringe and the syringe was then filled with the sample for pre-concentration. The solvent was dispersed using a magnetic stirring bar inside the syringe that was driven by an external rotating magnetic field. During the extraction, the sample was dispensed continuously using a low flow rate through the syringe void. After extraction, back-extraction to an aqueous acceptor phase was carried out and followed by spectrophotometric detection.

LIS operation mode, parameters of extraction including volume and type of the extraction solvent, flow rate and stirring rate, as well as parameters of back-extraction such as back-extraction solution type and concentration were optimized. The method was adopted for measurement of nitrophenols in surface water. Spectral analysis was applied for the quantification of the three isomers. The limits of detection for *o*-, *m*-, and *p*- nitrophenol (λ=400 nm) were 0.14, 0.26, and 0.02 µmol/L, respectively. Three samples with a volume of 26.5 mL could be extracted in one hour.

The major advantages of this technique in addition to typical characteristics of LIS are continuous operation mode, which allows the use of large sample volumes, and consequent achievement of high pre-concentration factors exceeding 50.

The study was supported by specific research No. 260 412/2017. The authors gratefully acknowledge the financial support of the project STARRS reg. No.: CZ.02.1.01./0.0/15_003/0000465 funded by ERDF.

TESTING OF ELECTROSPUN NANOFIBERS FOR ON-LINE EXTRACTION IN CHROMATOGRAPHY SYSTEMS

HÁKOVÁ M., RAABOVÁ H., CHOCHOLOUŠOVÁ HAVLÍKOVÁ L., ŠATINSKÝ D.

Department of analytical chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

e-mail: mhakova@faf.cuni.cz

Solid phase extraction (SPE) is an important part of sample analysis. Recent trends in SPE based methods are focused on finding of new sorbents and new approaches. Nanofibrous on-line SPE HPLC techniques fulfill both branches of modern trends in sample preparation. Nanofiber polymers have a great potential as sorbents due to their variability (chemical properties) and enhanced surface area (small fiber size) and on-line system ensures the repeatable and reproducible conditions of analysis with minimal demand on operator's skills.

Electrospun nanofibers are formed via electrospinning technology. Electrospinning, using strong electrostatic field for creation of polymer fibers from polymeric melt or solution, is elegant widely used technique for obtaining nanofibers. It can be used alone, creation of base polymeric nanofibers, or in combination with other fiber producing technology, creation of composite materials.

In this project various types of electrospun nanofibers were tested as new undescribed sorbents for on-line SPE HPLC determination of some biologically active substances (e.g. bisphenol A, ochratoxin A, carbaryl and some pyrethroids). Materials, included in this study were nylon 6, polyvinylidene difluoride (PVDF), polycaprolactone (PCL), polyethylene, and polystyrene nanofibers and some composite materials, mixture of nanofibers and microfibers (PCL/PVDF, PCL/PCL).

New on-line SPE HPLC methods using nanofibers as sorbents were developed, validated and applied on real samples (river water, soil and beer).

The study was supported by specific research, no. SVV 260 412 and GAČR project no. 17-08738S.

SEPARATION BEHAVIOR OF SELECTED PHENOLIC ANALYTES

HOLLÁ, M.,[1] SKLENÁŘOVÁ, H.,[1] ŠATÍNSKÝ, D.[1]

[1] Department of Analytical Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

e-mail: hollama@faf.cuni.cz

Phenolic compounds in different fruit types represent a group of substances with wide range of physico-chemical properties and contain at least one aromatic ring and more than one hydroxyl group. Phenolic compounds are mostly analyzed using reversed phase liquid chromatography with a gradient elution. The impact of different chromatographic conditions on separation efficiency was studied. Standard solution of main phenolic compounds including gallic acid, chlorogenic acid, epicatechin, rutin, phloridzin, quercetin, and phloretin, and methanolic apple extract were used in optimization.

The effects of mobile phase with pH values of aqueous component varying from 1.8 to 2.8, a column length of 100 and 150 mm, a particle size of 3 and 5 μm, type of particles including fully porous, core-shell, and multilayered, and columns packed with specially modified stationary phases for the separation of polar substances were tested. Mobile phase composed of acetonitrile and acidified aqueous component under the flow rate of 1 mL min^{-1} with gradients of different profiles were eventually tested for the separation of standard mixture and apple extracts.

The study was supported by specific research project No. SVV/260412/2017 and project of Technology Agency of the Czech Republic No. TAČR TJ01000151.

DEVELOPMENT AND OPTIMIZATION OF UHPLC-MS/MS METHOD FOR ANALYSIS OF HCV ANTIVIRALS IN SAMPLES ARISED FROM CELL EXPERIMENTS

PAVLÍK, J.,[1] MARTINEC, O.,[2] ČERVENÝ, L.,[2] KOČOVÁ VLČKOVÁ, H.,[1] SVOBODOVÁ, P.,[1] CHRENKOVÁ. L.,[1] NOVAKOVÁ, L.,[1]

[1] Department of Analytical Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

[2] Department of Pharmacology and Toxicology, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

e-mail: Pavlikja@faf.cuni.cz

Current clinical studies concerned with antiviral drugs show a great inter-individual variability of plasmatic concentrations even between patients with the same dosage. Antiviral drugs are known as substrates for some intestinal transporters important for their absorption and treatment effectivity. However, the common therapeutic monitoring is based on monitoring of viral load, not on the monitoring of the antiviral drug levels in plasma. So far, only few antiviral compounds have been monitored for their membrane transport through intestinal barrier or their membrane transport was not evaluated at all. For this purpose, the Caco-2 cells serve as common absorption model for orally administered drugs. Samples originated from these experiments are complex mixtures with a high content of compounds including salts, glucose, vitamins, and albumin, and are limited in volume to 50-200 μL.

In this study, tenofovir disoproxil fumarate (TDF) and its two metabolites (tenofovir, tenofovir monoester), sofosbuvir, and ledipasvir, were analyzed. Metabolites of TDF were analyzed due to quite fast metabolism and pharmacologic properties. The sofosbuvir and ledipasvir are commonly used in HIV therapy in combination with other drugs for their complementary effect. Different chromatographic modes such as RPxHILIC, mobile phase composition, additives, and stationary phases were tested during the optimization of method using the UHPLC-MS MS instrumentation. The compounds were detected in selected reaction monitoring mode with detailed optimization for each compound. Very important part of the analytical method was the sample preparation step. This step could not be accomplished via regular extraction due the

small volume of the sample; therefore, use of microextraction techniques was needed. The liquid-liquid microextraction was selected with the different extraction solvents optimization as the method of choice for the sofosbuvir. The best conditions for extraction, chromatography, and detection will be used for biological experiments and the method will be validated.

The study was supported by project GAUK 1600317, SVV 260 412 /2017 and by the STARSS project (Reg. No. CZ.02.1.01/0.0/0.0/15_003/0000465) co-funded by ERDF.

UHPLC-MS/MS STABILITY STUDY OF SOBUZOXANE AND ITS ACTIVE FORM - ICRF-154 IN BIOLOGICAL MATRICES

REIMEROVÁ, P.,[1] PISKÁČKOVÁ, H.,[1] JIRKOVSKÁ, A.,[2] ŠTĚRBOVÁ, P.[1]

[1] Department of Pharmaceutical Chemistry and Pharmaceutical Analysis, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

[2] Department of Biochemical Sciences, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

e-mail: reimerop@faf.cuni.cz

Inhibitor of topoisomerase II - sobuzoxane (SBZ) was synthesized as a prodrug of an anti-cancer agent ICRF-154 to improve its solubility and bioavailability. Furthermore, thanks to its structural similarity to dexrazoxane, the protective effect of SBZ on anthracycline-induced cardiotoxicity is under investigation. Although SBZ is approved as an anticancer drug in Japan, data on its bioactivation in biological matrices are scarce. Therefore, our aim was to conduct a bioactivation study of SBZ and ICRF-154 in several biological matrices and compare the results with our data collected on its close analogue - dexrazoxane (DEX).

SBZ (100 μM) was incubated with neonatal ventricular rat cardiomyocytes (NVCM), in the cell culture medium and in rabbit plasma for 24 hours. This experiment was followed by stability study of ICRF-154 and its open-ringed metabolite EDTA-diamide in the cell culture medium and plasma. For chromatographic assay, NVCM cells and plasma were treated with protein precipitation, cell culture medium was simply diluted. All analyses were performed on reversed-phase Zorbax SB-Aq column using Nexera UHPLC system coupled with LCMS-8030 triple quadrupole mass spectrometer with ESI ion source (Shimadzu). Mobile phase composed of 1 mM ammonium formate and methanol in a gradient mode provided the best separation of all analytes and corresponding internal standards in 13 min.

Inside NVCM cells very low concentrations of SBZ along with high concentrations of ICRF-154 were determined. ICRF-154 was then gradually metabolized to EDTA-diamide. The rapid degradation of SBZ was also observed in the cell medium without the NVCM and in rabbit plasma. The slower biodegradation of ICRF-154 was verified by an *in vitro* experiment. EDTA-diamide was stable in all tested media for 24 hours.

The study was supported by Charles University (projects GAUK 344 615 and SVV 260 401).

IS SUPERCRITICAL FLUID EXTRACTION USEFUL TOOL FOR EXTRACTION OF BIOACTIVE COMPOUNDS?

PILAŘOVÁ V., NOVÁKOVÁ L., ŠVEC F.

Department of Analytical Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

e-mail: pilarovv@faf.cuni.cz

Sample preparation plays a key role in modern analytical methods. It usually takes more than a 70% of time of the entire analytical process. Supercritical fluid extraction (SFE) is becoming very popular because it is environmentally friendly and can be used in wide range of applications. The advantages of this methods result from the properties of CO_2 that is used as the preferred extraction solvent. It has low viscosity and high diffusivity facilitating passage of extraction fluid through the solid sample. This property permits fast extraction in mere tens of minutes. Moreover, the change in CO_2 temperature and pressure that affects the solvent strength allows extraction of compounds with various polarity in different fractions. Polarity of CO_2 can be increased via addition of polar organic solvent such as methanol and ethanol. Use of CO_2 with a small volume of organic solvents allows the effective preconcentration of isolated compounds. SFE can be carried out in dynamic and static extraction operation modes where the flow-rate of solvent and time of extraction, both of which affect the mass transfer and the recovery of extraction, must be optimized. SFE can be readily automated and its on-line coupling with separation approaches represent the additional advantages. The contemporary SFE instrumentation allows the change of conditions during the extraction. Thus, the isolation of fractions containing biologically active compounds with various properties is possible to achieve in a single extraction run.

The study was supported by SVV 260 412 (2017), and the project STARSS reg. no.: CZ.02.1.01/0.0/0.0/15_003/0000465 funded by EFRR.

References:

1. DEL PILAR SÁNCHEZ-CAMARGO, A., PARADA-ALFONSO, F., IBANEZ, E., CIFUENTES, A.: J. Sep. Sci., 40, 2017, 213-227.
2. DA SILVA, R.P.F.F, ROCHA-SANTOS, T.A.P., DUARTE, A.C.: Trends Anal. Chem., 76, 2016, 40-51.

UHPLC-MS/MS OF A NOVEL DEXRAZOXANE ANALOGUE JAS-2 AND ITS PRODRUG

PISKÁČKOVÁ, H.[1] REIMEROVÁ, P.,[1] ROH, J.,[2] KARABANOVICH, G.,[2] ŠTĚRBOVÁ-KOVAŘÍKOVÁ, P.,[1]

[1] Department of Pharmaceutical Chemistry and Pharmaceutical Analysis, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

[2] Department of Organic and Bioorganic Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

e-mail: piskacha@faf.cuni.cz

Dexrazoxane (DEX) is the only approved, clinically used drug effective in protection of myocardium against anthracycline-induced toxicity. JAS-2 was synthesized as a novel analogue of DEX. Pilot studies indicate that JAS-2 is more effective in protection of neonatal rat cardiomyocytes from toxic effect of anthracyclines as compared with DEX. Due to the poor solubility of JAS-2 in water, a pro-drug with a code name - GK-667 was prepared. Bioanalytical method for investigation of GK-667, its conversion to the active form as well as further metabolism of JAS-2 is needed. The aim of this project is 1) to developed and validate UHPLC-MS/MS method for determination of GK 667, JAS-2 and its metabolite ($JAS2_{met}$) in cell culture medium - DMEM and 2) to apply it for the stability/activation study. The UHPLC system coupled with a triple quadrupole mass spectrometer with ESI^+ ion source was used (both, Shimadzu, Japan). The analysis was achieved on Luna Omega Polar column (100 x 3.0 mm, 2.5 μm) protected with a guard column. A mixture of ammonium formate and acetonitrile in a gradient mode was used as a mobile phase. DMEM samples were treated by dilution using water ($JAS2_{met}$) or 2% formic acid (JAS-2, GK-667). Method was validated according to FDA guideline (GK-667 and $JAS2_{met}$: 1-100 μmol/l, JAS-2: 5-100 μmol/l, $R^2 \geq 0.991$). Stability study on GK-667 and JAS-2 (100 μmol/l) was conducted in DMEM at 37°C *in vitro*. It shown that GK-667 is rapidly converted to JAS-2, which is slowly degraded to $JAS2_{met}$. In comparison with the conversion of DEX to its metabolite - ADR-925, JAS-2 degraded faster to its open-ringed metabolite.

The study was supported by the Charles University in Prague (projects GAUK 1550217 and SVV 260 401)

TESTING OF NANOFIBERS AS NOVEL SORBENTS USING SEQUENTIAL INJECTION ANALYSIS SYSTEM

ŠRÁMKOVÁ, I.,[1] CARBONELL ROZAS, L., [2] JIRSÍKOVÁ, [1] V., HORSTKOTTE, B., [1] ŠATÍNSKÝ, D. [1]

[1]Department of Analytical Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

[2]Department of Analytical Chemistry, Faculty of Sciences, University of Granada, Campus Fuentenueva s/n, E-18071 Granada, Spain

e-mail: sramkovai@faf.cuni.cz

Electrospun nanofibers (NF) have gained a lot of attention during the last years due to their unique features such as a large surface-to-volume ratio, possibility of using natural materials, and the possibility of customized chemical modification. Apart from their novel implementation e.g. in medicine, NF are currently in the focus of analytical chemists for their great potential as a sorbent in solid phase extraction (SPE) techniques.

Sequential injection analysis (SIA) system comprising a piston pump, a switching valve, and a suitable detector is an advantageous tool to study the potential of NF as extraction sorbent offering simple operation, flow manipulation, and fast results evaluation. In this work, we studied handling, packing, and use of different geometries and devices to engage NF in a SIA system for the first time. Both planar arrangements and column format were studied. A specially designed 3D-printed holder allowing to house a single sheet of NF was chosen as the most suitable device due to the low amount of NF required, low backpressure, and minimized dead volume compared to the column format.

The extraction capacities of polyvinylidene fluoride, polyamide, polystyrene, polylactic acid, polycaprolacton and polyacrylonitrile NF were tested with model analytes differing in their physical-chemical properties. Retention of the molecules was evaluated from peak height measurements and the results will be presented.

This work was supported by the STARSS project (Reg. No. CZ.02.1.01/0.0/0.0/15_003/0000465) co-funded by ERDF. The authors also acknowledge the support of the Czech Science Foundation through project No. 17-08738S.

SEQUENTIAL INJECTION DETERMINATION OF THE HERBICIDE 2,4-DICHLOROPHENOXYACETIC ACID USING PRECONCENTRATION WITH A POLYMER INCLUSION MEMBRANE

ZELENÁ, L.[1,2], ALMEIDA, M.I.G.S.[2], CATTRALL, R.W.[2], SKLENÁŘOVÁ, H.[1], SOLICH, P.[1], KOLEV, S.D.[2]

[1] Department of Analytical Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

[2] School of Chemistry, Faculty of Science, The University of Melbourne, Australia

e-mail: zelenal@faf.cuni.cz

A sequential injection analysis (SIA) system for the determination of the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) in natural waters, which uses a polymer inclusion membrane (PIM) with 20% Aliquat 336 as the carrier, 10% 1-tetradecanol as a modifier, and 70% poly(vinyl chloride) as the base polymer for on-line analyte extraction and preconcentration has been developed. PIMs are extracting liquid membranes that offer improved stability compared to supported liquid membranes. They have been used successfully in the past for on-line extraction in flow injection analysis [1-3]. After preliminary testing using batch-conditions, the SIA system parameters have been optimized and will be validated with the analysis of water samples.

The study was supported by the STARSS project CZ.02.1.01/0.0/0.0/15_003/0000465, co-funded by ERDF, and by the Mobility Fund of the Charles University.

References

1. ZHANG, L.L., CATTRALL, R.W., KOLEV, S.D.: Talanta 84 (2011) 1278-1283.

2. ZHANG, L.L., ASHOKKUMAR, M., CATTRALL, R.W., KOLEV, S.D.: Talanta 97 (2012) 382-387.

3. NAGUL, E.A., FONTÀS, C., McKELVIE, I.D., CATTRALL, R.W., KOLEV, S.D.: Anal Chim Acta 803 (2013) 82-90.

CANDIDA ALBICANS METABOLOMICS AND QUORUM SENSING IN HUMAN VAGINAL SWABS USING HIGH-RESOLUTION MASS SPECTROMETRY

JAKUBEC P.[1], VLČKOVÁ H. [1], PILAŘOVÁ V. [1], BUCHTA V. [2], NOVÁKOVÁ L.[1]

[1] Department of Analytical Chemistry Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

[2] Department of Clinical Microbiology, University Hospital and Faculty of Medicine in Hradec Králové, Czech Republic

e-mail: jakubep1@faf.cuni.cz

Candida albicans (CA) is a common part of human microbiota, typically present in oral cavity, gastrointestinal tract, and vagina. The quorum sensing is a system, which regulates population density of the microorganisms and enables them to respond to changing physiological and environmental factors including human body. In case of CA quorum, sensing molecules are represented with farnesol and tyrosol. In general, farnesol blocks the morphogenic switch from yeast to hyphae and tyrosol supports it.

Vaginal swabs were collected and diluted in phosphate buffer saline solution. Total of 68 patients and 14 healthy controls with and without vulvovaginal discomfort were analyzed. Presence of CA and its morphotypes (blastoconidia versus pseudo/hyphae) and culture positivity were also defined.

The study aimed at correlation of metabolomic data to microbiological evaluation of chronic vulvovaginal discomfort. Firstly, the MS full scan spectra were used for identification of features detected using the following parameters: minimum peak width 2 s, signal to noise ratio larger than 10, and <5 ppm mass accuracy. Secondly, the principal component analysis (PCA) of individual patients, CA negative control, and pooling of samples were carried out. PCA of previously detected features and their dysregulation were distinguished as clusters, which correlated to microbiological parameters including pH, Nugent score, co-existence of different bacteria such as Gram negative *Coccobacillus* and *Lactobacillus*, and presence of *Candida albicans.*

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, SVV 260 412/2017 and Ministry of Health of the Czech Republic grant nr. 15-29225A.

DEVELOPMENT OF CAPILLARY ELECTROPHORESIS METHOD FOR THE CHARACTERIZATION OF PHARMACEUTICALS CONTAINING SILYMARIN COMPLEX

RIASOVÁ, P., JÁČ, P., POLÁŠEK, M.

Department of Analytical Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

e-mail: riasovpe@faf.cuni.cz

The project is focused on the development and application of capillary electrophoretic (CE) method allowing separation of structurally similar *Silybum marianum* flavonolignans, namely: silybin A (SBA), silybin B (SBB), isosilybin A (ISBA), isosilybin B (ISBB), silychristin (SCH), silydianin (SD) and their precursor taxifolin (TX) occurring in Silymarin complex.

The method development involved optimization of a number of experimental conditions such as concentration of boric acid, type and concentration of cyclodextrins, volume fraction of organic modifier, pH of the background electrolyte and length of capillary. Optimal background electrolyte was: 100 mM boric acid of pH 9.0 (adjusted with 1 M NaOH), 5 mM heptakis(2,3,6-tri-*O*-methyl)-β-cyclodextrin and 10% (v/v) methanol. The separation was carried out in a fused silica capillary (internal diameter 50 μm, total length 80.5 cm and effective length 72 cm), with applied voltage 25.0 kV and UV detection at 200 and 320 nm. Base-line separation of all flavonolignans, including diastereomers SBA/SBB and ISBA/ISBB with resolution 1.73 and 2.59, respectively, was attained. The method was validated and subsequently applied to CE analysis of dietary supplements and a drug containing the silymarin complex.

The study was supported by SVV 260412/2017.

UHPLC-MS/MS ANALYSIS OF ANTIVIRAL AGENT SOFOSBUVIR FOR STUDY OF ITS TRANSPORT MECHANISM

SVOBODOVÁ, P., [1] SYŘÍNEK J. E., [1] ŠEMLEJ T., [1] MARTINEC O., [2] PAVLÍK J., [1] KOČOVÁ VLČKOVÁ H., [1] SOLICH P., [1] NOVÁKOVÁ L., [1]

[1] Department of Analytical Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

[2] Department of Pharmacology and Toxicology, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

e-mail: svobodopavl@faf.cuni.cz

The antiviral agents are often administered in combination pharmacotherapy. Therefore, their administration increases the risk of drug-drug interactions on ABC transporters that can potentially lead to failure of the treatment. For this reason, the understanding of the ABC transporters-mediated drug-drug interaction is important for safety and effectivity of the therapy. The aim of this project was the study of drug-drug interaction of sofosbuvir with other antiviral agents. Transport experiments were based on two buffer compartments, i.e. Hank's Balance Salt Solution, pH 6.5 or 7.4 with 1% albumin, separated by the Caco-2 cells monolayer, which served as the transport medium for the drug. The main analytical assignment was to develop and validate method for the determination of sofosbuvir in the buffer compartments.

Liquid-liquid extraction was used for the sample preparation with butyl acetate as the optimal extraction solution. Sofosbuvir was quantified using ultra high-performance liquid chromatography coupled with tandem mass spectrometry in the selected reaction monitoring (SRM) mode. The optimized chromatographic analysis in ACQUITY UPLC BEH HILIC 2.1×100 mm (1.7 μm) column was carried out in 3 min long isocratic HILIC elution with the mobile phase comprising 80:20 acetonitrile and 10mmol/L ammonium acetate pH 6.0 at a flow rate of 0.3 mL min^{-1}. Our method was validated and subsequently applied for the samples obtained from the transport experiments. The results confirmed the presence of efflux transporters. In future, interactions of sofosbuvir with other antiviral drugs will be evaluated. The selectivity of UHPLC-MS/MS method will be improved by using isotopically labeled

internal standards and by further optimization of separation to achieve separation of coeluting antiviral agents.

The study was supported by the STARSS project (Reg. No. CZ.02.1.01/0.0/0.0/15_003/0000465), the Project of Specific research, SVV 260412/2017 and the project GAUK 1600317.

Pathobiochemistry and Xenobiochemistry

GPXS, MICRORNA AND OBESITY ASSOCIATION

HANOUSKOVÁ, B., MATOUŠKOVÁ, P.

Department of Biochemical Sciences, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

e-mail: hanouskb@faf.cuni.cz

Obesity is a serious health problem worldwide and is associated with increased risk of chronic diseses such as cardiovascular diseases, metabolic syndrome, and diabetes mellitus. The finding of the molecular mechanisms involved in adipogenesis could lead to the identification of novel biomarkers and therapeutic targets in treatment of obesity and potentially developing of anti-obesity drugs. Excessive cellular acummulation of reactive oxygen species (ROS) causes cell damage and is involved in a wide range of human diseases, including obesity and obesity-related pathologies. Cellular antioxidant system, that maintains the cellular balance of ROS, consists of non-enzymatic and enzymatic systems, including glutathione peroxidases (GPxs). GPxs, catalyze the reduction of H_2O_2 or organic hydroperoxides to water or the corresponding alcohols, and act also as sensors to transfer the message to its interacting proteins. Results of many studies showed that knockout of some GPx has fatal consequences, and changes in GPx expression are connected with severe pathologies, including obesity and diabetes. Therefore, findings of the regulatory mechanisms involved in GPxs expression are of a great importance. The one of significant way in regulation of GPxs expression are microRNAs (miRNAs). MiRNAs are small non-coding, single-stranded RNA molecules consist of about 22 nucleotides responsible for the negative posttranscriptional regulation of a variety human genes, hybridize mostly to 3´untranslated region. Among other, the abberant expression of miRNAs may be associated with some disorders and diseases. In this study we decided to reveal the possible association between GPxs, microRNAs, and obesity and observe the effect of excessive fructose in the diet. For our experiment, we used standard high fat diet obesity mice model with or without fructose administration. We observed changes in the expression of GPxs and selected miRNAs, in liver and three types of adipose tissue (brown, visceral, and subcutaneous).

References:

1. BARTEL DP. Micrornas: target recognition and regulatory functions. Cell. 2009; 136: 215-233.
2. UFER C & WANG CC The roles of glutathione peroxidases during embryo development. Frontiers in molecular neuroscience. 2011.

ACUTE TOXICITY OF R-PULEGONE AND R-MENTHOFURAN IN HUMAN LIVER SLICES

ZÁRYBNICKÝ, T., [1] LANCOŠOVÁ, B., [1] ŠUBRT, Z., [2] BOUŠOVÁ, I. [1], SKÁLOVÁ, L. [1]

[1]Department of Biochemical Sciences, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

[2] Department of Surgery, Charles University in Prague, Faculty of Medicine, Šimkova 570, Hradec Králové, Czech Republic

e-mail: zarybnto@faf.cuni.cz

Monoterpenes R-pulegone (PUL) and R-menthofuran (MF) are major constituents of several plants and essential oils (e.g. peppermint, pennyroyal) used for flavoring foods and drinks, for herbal medicinal products and cosmetics. MF is the major metabolite of PUL in the body and they both display similar hepatotoxicity in rodents. Exposure to PUL and MF is primarily through food products and beverages flavored with spearmint oil, peppermint oil, or synthetic PUL. Serious/lethal cases of intoxication from pennyroyal oil with a high content of PUL indicated first that it is a potent hepatotoxin. Tolerable daily intake for PUL and MF has been set for food at 0.1 mg/kg bw however, doses exceeding that are commonly encountered in herbal medicinal products. [1] Despite a large number of PUL and MF toxicity and metabolism studies, vast majority of them are limited to rodents, making it difficult for regulatory authorities to apply the information to humans. In our experiments, 5 human liver samples received from surgery were used to gain precision-cut liver tissue slices, which were cultivated for 24 hours in the presence of PUL and MF to determine acute toxicity in humans *ex vivo*. The half maximal inhibitory concentration (IC_{50}) for PUL and MF was determined to be 293 μM and 418 μM, respectively. We are planning to also determine influence of these hepatotoxicants on expression of liver enriched miRNAs (e.g. 122-5p, 885-5p, 192-5p, 125b-5p), since there is to our knowledge no such a study validating liver slices on miRNA level.

The study was supported by by the Czech Science Foundation (grant No. P303/12/G163)

References

1. EUROPEAN MEDICINES AGENCY - EMA/HMPC/138386/2005 REV. 1 [online]. Dostupné z: http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2016/07/WC500211079.pdf

IN VITRO STUDY OF DEXRAZOXANE ANALOG JAS-2 AND ITS WATER-SOLUBLE PRODRUGS

JANSOVA, H.,[1] JIRKOVSKA, A.,[1] KARABANOVICH, G.,[2] ROH, J.,[2] SIMUNEK, T.,[1]

[1] Department of Biochemical Sciences, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

[2] Department of Organic and Bioorganic Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

e-mail: jansovah@faf.cuni.cz

Anthracyclines (ANT) such as daunorubicin or doxorubicin are among the most effective anticancer drugs, but their use is hampered by their irreversible cardiotoxic side effects. 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.[1] Latest studies suggest that its activity is caused by catalytic inhibition of topoisomerase II.[2] However, its use in clinical practice is limited due to its high price and potential to increase some side effects of ANT (e.g. myelotoxicity). Therefore, in this work we prepared and studied DEX analog JAS-2 and, due to its poor solubility in water, also soluble JAS-2 prodrugs.

JAS-2 as well as its prodrugs significantly protected isolated neonatal rat cardiomyocytes against toxicity induced by daunorubicin. Especially in lower concentrations, their effectiveness was significantly higher than the effect of DEX. Whereas they are topoisomerase II inhibitors and act on the same enzyme as ANT, we also studied their impact on cell proliferation and ANT antiproliferative activity. All studied compounds showed significant antiproliferative activity in HL-60 leukemic cell line also better than DEX. None of studied compounds compromised the antiproliferative effect of daunorubicin, thus they can be further developed as potential cardioprotective agents against ANT-induced cardiotoxicity.

The study was supported by Czech Science Foundation (18-08169S) and PROGRES (160/11/1107-2).

References

1. VAN DALEN, E.C., CARON, H.N., DICKINSON, H.O., KREMER, L.C.: Cochrane Database Syst Rev., 2011, Cd003917.

2. LYU, Y.L., KERRIGAN, J.E., LIN, C.P., AZAROVA, A.M., TSAI, Y.C., BAN, Y., LIU, L.F., Cancer Res., 67, 2007, 8839-46.

ROLE OF HEXOSE-6-PHOSPHATE IN PROLIFERATION AND MIGRATION OF CANCER CELLS

NAVRATILOVA, H.,[1] TSACHAKI, M.,[2] ODERMATT, A.,[2]

[1] Department of Biochemical Sciences, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

[2] Division of Molecular and Systems Toxicology, Department of Pharmaceutical Sciences, University of Basel, Switzerland

e-mail: stamh4aa@faf.cuni.cz

Hexose-6-phosphate dehydrogenase (H6PD) produces reduced NADPH in the endoplasmic reticulum (ER) lumen. NADPH constitutes a cofactor for many reducing enzymes. The H6PD gene is amplified in several types of malignancies, and earlier work pointed toward a potential involvement of the enzyme in cancer cell growth. In the present study, a pivotal role of H6PD in proliferation and migratory potential of 3 human breast cancer cell lines was demonstrated. Knockdown of H6PD decreased proliferation and migration in SUM159, MCF7, and MDA-MB-453 cells. To understand the mechanism through which H6PD exerts its effects, the cellular changes after H6PD silencing in SUM159 cells were investigated. Knockdown of H6PD resulted in an increase in ER lumen oxidation, and down-regulation of many components of the unfolded protein response, including the transcription factors activating transcription factor-4, activating transcription factor-6, split X-box binding protein-1, and CCAAT/enhancer binding protein homologous protein. This effect was accompanied by an increase in sarco/endoplasmic reticulum Ca^{2+}-ATPase-2 pump expression and an decrease in inositol trisphosphate receptor-III, which led to augmented levels of calcium in the ER.

The study was supported by PROGRES Q42 and Swiss National Science Foundation Grants 31003A-159454.

References

1. LAVERY, G. ET AL.: J. Biol. Chem. 283, 2008, 8453–61.
2. MARINI, C. ET AL.: Sci. Rep. 6, 2016, 25092
3. KNEZEVIC, C. E. ET AL.: Cell Chem. Biol. 23, 2016, 1–14.

EVALUATION OF DRUG UPTAKE AND DEACTIVATION IN PLANT

RAISOVÁ STUCHLÍKOVÁ, L.,[1] PODLIPNÁ, R.,[2] SZOTÁKOVÁ, B.,[1] SKÁLOVÁ, L.,[1]

[1] Department of Biochemical Sciences, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

[2] Laboratory of Plant Biotechnology, Institute of Experimental Botany, Czech Academy of Science, Czech Republic

e-mail: lucieraisova@faf.cuni.cz

Veterinary pharmaceuticals are used in large amounts in modern husbandry for treatment and prevention of diseases in animals. These drugs represent important source of environmental pollution as they can reach environment through the treatment processes, inappropriate disposal of used containers, unused medicine or livestock feed, and manufacturing processes. Plants are exposed to veterinary pharmaceuticals in pastures with treated animals, in fields fertilized with dung from treated animals or in aquatic ecosystems. Several reviews have been published regarding the potential impact of pharmaceuticals on plants. Pharmaceutical as well as other xenobiotics enter plant body and can induce stress and consequent response. Anyway, in plants as well as in all other organisms, each xenobiotic represent a potential risk. Therefore, a sophisticated defence system in the form of xenobiotic-metabolizing enzymes exist. Owing these enzymes, plants are able to transform xenobiotics into various metabolites and store them in the vacuoles and cell walls (Bartikova et al. 2016). Generally, the metabolites are non-toxic or less-toxic, but some metabolites can be similar or even more toxic than their parent compound (Halling-Sørensen et al. 2002). For this reason, more detailed information about the metabolic pathways of each xenobiotic is necessary for a complex evaluation of eco-toxicological risks.

The study was supported by Czech Science Foundation No. 15-05325S *and PROGRES Q42.*

References

1. BÁRTÍKOVÁ, H., PODLIPNÁ, R., SKÁLOVÁ, L.: Chemosphere, 144, 2016, 2290–2301.
2. HALLING-SORENSEN, B., SENEGELOV, G., TJORNELUND, J.: Archives of Environmental Containation and Toxicology 42, 2002, 263–271.

Pharmacology and Toxicology

OXYSTEROLS AFFECT ENDOGLIN EXPRESSION, INFLAMMATION AND DEVELOPMENT OF ENDOTHELIAL DYSFUNCTION IN HUMAN AORTIC ENDOTHELIAL CELLS

VICEN, M.[1], HAVELEK, R.[2], DOLEZELOVA, E.[1], PRASNICKA, A.[1], VITVEROVA, B.[1], NACHTIGAL, P.[1]

[1] Department of Biological and Medical Sciences, Faculty of Pharmacy in Hradec Kralove, Charles University, Czech Republic

[2]Department of Medical Biochemistry, Faculty of Medicine in Hradec Kralove, Charles University, Czech Republic

Email: vicenm@faf.cuni.cz

Endoglin (CD105, TGF-βRIII receptor), acts as auxiliary partner protein in TGF-β receptor complex. This protein is essential for endothelial NO synthesis and proper endothelial function. On the other hand, increased expression of endoglin was shown to participate in inflammatory infiltration of leukocytes and development of endothelial dysfunction. In addition, previous *in vivo* studies showed that hypercholesterolemia affects membrane and soluble endoglin expression/levels, however the precise importance/consequence of this effect is unknown. In this study, we aimed to elucidate the effects of 7-Ketocholesterol (oxysterol with non-enzymatic origins, 7K) and 22-Hydroxycholesterol (oxysterol with enzymatic origins, 22OH) on the development of endothelial dysfunction and expression of endoglin with respect to role of endoglin in the development of endothelial dysfunction in human aortic endothelial cells (HAEC).

HAECs passage 5 were exposed to 7K or 22OH (5,10ug/mL) for 12 hours. Gene activity was evaluated using qRT-PCR and protein expression using flow cytometry (direct, indirect or intracellular). We demonstrated that only oxysterol with non-enzymatic origins – 7K was able to significantly increase expression of cell adhesion molecules and endoglin on gene, but also protein levels. These results suggest potential involvement of endoglin in endothelial dysfunction development after 7K treatment. Therefore, we focused on regulation of endoglin expression via 3 main transcription factors – KLF6, LXR and NF-κB pathway. We found out,

that inhibition of either KLF6 or LXR pathway is able to prevent 7K induced increase in endoglin expression.

We demonstrated that 7K is able to affect endoglin expression, its signaling and inflammation in endothelial cells. However, precise importance of endoglin and its regulation with respect to potential protective or harmful effects on endothelium is investigated in our lab.

Grants: This work was supported by grants from Czech Science Foundation (GACR 15-24015S), Grant Agency of Charles University GAUK 1158413C, GAUK 1216217 and SVV/2017/260414.

HIGH SOLUBLE ENDOGLIN LEVELS ACCOMPANIED BY MILD HYPERCHOLESTEROLEMIA AGGRAVATE ENDOTHELIAL DYSFUNCTION IN MOUSE AORTA

VITVEROVA, B., [1] BLAZICKOVA, K., [1] NAJMANOVA, I., [1] VICEN, M., [1] PERICACHO, M., [2] NACHTIGAL, P. [1]

[1] Department of Biological and Medical Sciences, Faculty of Pharmacy in Hradec Kralove, Charles University, Czech Republic

[2] Renal and Cardiovascular Physiopathology Unit, Department of Physiology and Pharmacology, University of Salamanca, Salamanca, Spain

Email: vitverob@faf.cuni.cz

Soluble endoglin (sEng) is generated by the cleavage of the extracellular domain from membrane-bound endoglin in endothelial dysfunction-related pathologies, such as atherosclerosis. With respect to hypercholesterolemia as a risk factor of endothelial dysfunction, we hypothesized that combination of high sEng levels and hypercholesterolemia will results in the development/aggravation of endothelial dysfunction.

Three-month-old female transgenic mice on CBAxC57BL/6J background with high levels of sEng (Sol-Eng$^+$ *high*) and their littermates with low levels of sEng (Sol-Eng$^+$ *low*) were fed high fat diet for six months. We analyzed sEng levels (ELISA), total cholesterol levels and inflammatory markers (LUMINEX) in blood. Functional parameters of vascular reactivity was measured by wire myograph. Western Blot analysis of protein expression in aorta was performed.

Functional analysis of aorta showed impaired KCl induced vasoconstriction, endothelial-dependent relaxation after administration of acetylcholine as well as endothelial-independent relaxation induced by sodium nitroprusside in *Sol-Eng$^+$ high* group compared to *Sol-Eng$^+$ low* group. The expressions of membrane endoglin, p-eNOS/eNOS, pSmad2/3/Smad2/3 signaling pathway affecting vascular properties of aorta were significantly lower in *Sol-Eng$^+$ high* group compared to *Sol-Eng$^+$ low* group without significant effect on vascular inflammation markers (VCAM-1, ICAM-1, P-selectin and NF-κB).

The results indicate that long-term hypercholesterolemia combined with high levels of sEng leads to the aggravation of endothelial dysfunction with possible alteration of membrane endoglin/eNOS signaling suggesting that high levels of sEng might be considered as a risk factor of cardiovascular diseases.

Grants: The study was supported by grant from The Grant Agency of Charles University in Prague number 884216/C and grant SVV/2017/260414. This work was supported by grant from Czech Health Research Council AZV CR number 17-31754A.

PHARMACOKINETIC INTERACTIONS OF NOVEL ANTICANCER DRUGS WITH ABC DRUG EFFLUX TRANSPORTERS AND CYTOCHROMES P450

VAGIANNIS, D., HOFMAN, J., ČEČKOVÁ, M., ŠTAUD, F.

Department of Pharmacology and Toxicology, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

e-mail: vagiannd@faf.cuni.cz

Cancer treatment often fails due to multidrug resistance (MDR) of cancer cells. Possible causes of pharmacokinetic MDR include increased efflux of cytostatic drugs from the cell by ATP-binding cassette (ABC) drug efflux transporters and/or deactivation by drug metabolizing enzymes such as cytochromes P450 (CYP450s). Both of these mechanisms can lead to decreased concentrations of a cytostatic below the cytotoxic level. In the present work, we investigated interactions of nine new small molecule targeted drugs (alisertib, talazoparib, entinostat, entrectinib, onalespib, sapanisertib, tepotinib, ensartinib and vistusertib) with ABC transporters and CYP450s that are recognized perpetrators of pharmacokinetic MDR, i.e. with ABCB1, ABCG2, ABCC1 and CYP3A4, CYP3A5, CYP2C8, respectively. Tested compounds represent promising candidates from several pharmacological groups currently undergoing late phases (II/III) of clinical evaluation for lung and/or breast cancers, the unshakeable leading killers within oncological diseases. Inhibitory properties of selected drugs toward examined ABC transporters were tested using hoechst 33342 and calcein AM accumulation/efflux methods in MDCKII-ABCB1, MDCKII-ABCG2 and MDCKII-ABCC1 cell lines transduced with respective human transporters. We observed significant dual inhibition of ABCB1 and ABCG2 by onalespib, tepotinib and ensartinib. In addition, tepotinib as well as alisertib were demonstrated to be also ABCC1 inhibitors. CYP450 inhibition was assessed using commercial Vivid CYP450 screening kits. In these experiments, we found entinostat as the multiple inhibitor of CYP3A4, CYP3A5 and CYP2C8 while entrectinib inhibited tested CYP450 isoforms with the exception of CYP2C8. Obtained screening results will be confirmed with another appropriate methods. Verified inhibitors will be further evaluated in combination experiments and their ability to potentiate the cytotoxic effect of MDR-victim cytostatics will be determined. In conclusion, our results serve as an important starting point for future studies, which might reveal possible beneficial therapeutic strategy.

The study was supported by Czech Science Foundation, project No. 16-26849S and SVV/2017/260-414.

THE IN VITRO AND EX VIVO TESTING OF ANTIVIRAL DRUGS EFFECT ON RHODAMINE123 INTESTINAL ABSOPTION

MARTINEC O., ČERVENÝ L., VOKŘÁL I., ŠTAUD F.

Department of Pharmacology and Toxicology, Faculty of Pharmacy in Hradec Kralove, Charles University, Czech Republic

e-mail: martinon@faf.cuni.cz

ATP-binding cassette (ABC) efflux transporters including highly expressed P-glycoprotein (ABCB1) and Breast Cancer Resistance Protein (ABCG2) are known to reduce intestinal absorption of many orally administered drugs. Drug-drug interactions (DDIs) on these transporters can result in changes in levels of plasma concentrations leading frequently associated with decreased efficacy or safety of therapy.

As DDIs on ABCB1 or ABCG2 transporters cannot be investigated directly in humans, surrogate techniques has to be used. In our project we employed *in vitro* bidirectional transport experiments in colorectal adenocarcinoma cells Caco-2 cell line and *ex vivo* precision cut intestinal slices (PCIS) from rat ileum and we tested potency of selected antiviral drugs (lopinavir, ritonavir, abacavir, zidovudine, tenofovir disoproxil fumarate, rilpivirine, saquinavir, atazanavir, maraviroc, etravirine, ledipasvir, daclatasvir, sofosbuvir) to inhibit ABCB1-/ABCG2-mediated transport of fluorescent ABCB1/ABCG2 model substrate rhodamine123 (RHD123).

Lopinavir, ritonavir, and saquinavir significantly abolished transport of RHD123 across monolayers of Caco-2 cells and RHD123 efflux from PCIS while atazanavir and daclatasvir revealed significant effect only in one model used; in Caco-2 cells and PCIS, respectively.

In conclusion, we further confirmed that reported inhibitors lopinavir, ritonavir, and saquinavir have potential to increase intestinal absorption of ABCB1/ABCG2 substrates. Moreover, method of accumulation in PCIS that combines advantages of experiments *in vivo* with high throughput capability of *in vitro* systems was shown as suitable model for screening/verifying the DDIs on ABCB1/ABCG2 transporters, however, interspecies differences should be always considered.

This work was supported by SVV 260414/2017 and GAUK 1600317.

ENTECAVIR TRANSPORT ACROSS PLACENTA AND THE ROLE OF NUCLEOSIDE TRANSPORTERS

KARBANOVA S., ČERVENÝ L., ŠTAUD F.

Department of Pharmacology and Toxicology Faculty of Pharmacy in Hradec Králové, Charles University in Prague, Czech Republic

e-mail:karbanos@faf.cuni.cz

Entecavir is a highly used hydrophilic nucleosid-derived antiviral drug exhibiting high efficacy against hepatitis B virus (HBV). As there is lack of safety data and information about mechanism involved in entecavir placental transfer, its use during pregnancy is limited. Entecavir is suggested substrate of nucleoside transporters (NTs) that are categorized in two subfamilies – equillibrative nucleoside transporters (ENTs) and concentrative Na^+ dependent nucleoside transporters (CNTs). ENTs subtypes can be further distinguished by the sensitivity to S-(4-Nitrobenzyl)-6-thioinosinu (NBMPR).

We aimed to determine whether NTs participate in transplacental passage of entecavir. For this purpose we employed an *in vitro* uptake experiment in BeWo cells at 37°C and 4°C and an *in situ* dually perfused rat term placenta model (open-circuit), analyzing materno-fetal (M-F) and feto-maternal (F-M) transplacental clearances of entecavir on the organ level.

Using BeWo cell line model we observed the effect of all inhibitors with significant decrease of [^{3}H]entecavir intracellular concentration in presence of 100 μM NBMPR, 5 mM uridine and 1 mM adenosine. [^{3}H]entecavir M-F and F-M clearances showed low level of its transplacental permeation, with negligible placental accumulation after the perfusion (≤3% of the [^{3}H]entecavir dose). We observed significant discrepancy between M-F and F-M compartments. NBMPR (100 μM) decreased entecavir total clearance significantly in both directions, whereas in M-F direction the significance appeared also in presence of 0.1 μM NBMPR and similarly in presence 5 mM uridine.

In conclusion, our data suggest involvement of ENTs and CNTs in transplacental permeation of entecavir. Further studies have to be performed to specify the subtypes of ENTs or CNTs involved in entecavir placental transfer and propensity of entecavir to drug-drug interactions on these carriers.

The study was supported by GAUK 324215/C/2015 and SVV/ 2017/260-414.

REFERENCES

1. T. YAMAMOTO, K. KUNIKI, Y. TAKEKUMA, T. HIRANO, K. ISEKI, AND M. SUGAWARA, “Ribavirin uptake by cultured human choriocarcinoma (BeWo) cells and Xenopus laevis oocytes expressing recombinant plasma membrane human nucleoside transporters.,” Eur. J. Pharmacol., vol. 557, no. 1, pp. 1–8, Feb. 2007.
2. Z. MA, X. YANG, T. JIANG, M. BAI, C. ZHENG, D. SUNG, H.JIANG, “Multiple SLC and ABC transporters contribute to the placental transfer of entecavir.,“ Drug metab. Dispos., Jan. 2017.

THE INHIBITORY EFFECT OF ANTIRETROVIRAL DRUGS ON THE TRANSPORT OF L-CARNITINE IN HUMAN PLACENTA

KARAHODA, R., ČEČKOVÁ, M., ŠTAUD, F.

Department of Pharmacology and Toxicology, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

e-mail: karahodr@faf.cuni.cz

Fetal L-carnitine levels, a cofactor in fatty acid beta-oxidation, are predominantly maintained through the materno-fetal placental transport. The uptake of L-carnitine to the placental trophoblast layer is ensured through the Na^+-dependent, high-capacity organic cation/carnitine transporter 2 (OCTN2), largely expressed in the apical plasma membrane of syncytiotrophoblasts. Affected transplacental transfer of carnitine can cause carnitine deficiency in fetus that is believed to be associated with pathological conditions, such as cardiomyopathy, skeletal myopathy, hypoketotic hypoglycemia etc.

The aim of our study was to investigate the inhibitory potential of a broad range of antiretroviral drugs on the OCTN2-mediated uptake of L-carnitine in human placenta. This was achieved by employing the *in vitro* approach in choriocarcinoma cell line BeWo and *ex vivo* techniques of L-carnitine uptake into the fresh villous fragments and microvillous plasma membrane vesicles (MVM), both isolated from human term placentas. The drugs included in the study comprised those targeting the human immunodeficiency virus enzymes, specifically: reverse transcriptase, proteases (PIs) and integrase; as well as viral entry inhibitors.

The initial screenings in BeWo cells revealed all tested PIs as inhibitors of L-carnitine uptake. Subsequent uptake experiments in *ex vivo* models confirmed the significant inhibitory potency of the protease inhibitors ritonavir (10 μM) and saquinavir (10 μM), causing decrease of the L-carnitine uptake by 31% and 38% in MVM vesicles and 44% and 35% in placental fresh fragments, respectively. Further evaluation will be needed to confirm the impaired transplacental carnitine caused by protease inhibitors in clinical settings and verify its impact on the fetal health in order to help optimize pharmacotherapy in pregnancy.

The study was supported by the Grant Agency of Charles University (Grant No. GAUK 1574217/C/2017).

NUCLEOSIDE TRANSPORTERS; ROLE IN PHARMACOKINETICS AND MECHANISMS OF REGULATION

JIRASKOVA L[1]., CERVENY L[1]., KARBANOVA S[1]., RYSKA A[3]., HORNYCHOVA H[3], CECKA F[4], TEBBENS E.J.D[2]., CECKOVA M[1]., PTACKOVA Z[1]., KARAHODA R[1]., STAUD F[1].

[1]Department of Pharmacology and Toxicology Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

[2]Department of Biophysics and Physical Chemistry Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

[3]The Fingerland Department of Pathology, Faculty of Medicine and University Hospital Hradec Kralove, Charles University, Czech Republic

[4]Department of Surgery, Faculty of Medicine and University Hospital Hradec Kralove, Charles University, Czech Republic

e-mail: jiraskl2@faf.cuni.cz

Nucleoside transporters (NTs) are ubiquitously occurring proteins categorized into two subfamilies: equilibrative nucleoside transporters (ENT1, ENT2) and concentrative nucleoside transporters (CNT2, CNT3). They are predominantly needed for maintaining nucleoside homeostasis and exhibit signifiant role in pharmacokinetics of nucleoside-derived drugs. In our research, we aimed to elucidate expression profile of NTs in the placental tissues, mechanisms of gene expression regulation and to investigate whether they contribute to placental pharmacokinetics of nucleoside-derived antivirals and to pharmacoresistance of pancreatic cancer. We found that ENT1 and CNT2 are dominant subtypes of ENTs and CNTs, respectively, in first-/third- trimester human placenta, rat term placenta, and BeWo cells. Both subfamilies revealed considerable inter-individual differences in all types of tested tissues and CNT2 exhibited intraindividual variability in human placentas, increasing its expression in the course of gestation. In the follow-up study, we showed that this increase might be associated with induced activity of cAMP/protein kinase A pathway. Concerning placental pharmacokinetics, we observed that NTs significantly contribute to placental transfer of anti-HBV entecavir, anti-HCV ribavirin, and anti-HIV abacavir. As ENT1 was suggested as

predictive biomarker of susceptibility of patients with pancrearic cancer to gemcitabine, we performed retrospective study that, however, did not confirm the reported hypothesis.

In conclusion, our findings have significantly broadened knowledge about placental expression of NTs, their regulation, and role in drug pharmacokinetics, in particular in distribution of nucleoside-derived antivirals into fetal circulation and pharmacoresistance of pancreatic cancer.

The study was supported by GAUK 812216/C/2016, GAUK 324215/C/2015 and SVV/2017/260-414.

RADIOLABELED MONOCLONAL ANTIBODY RAMUCIRUMAB: IN VITRO AND IN VIVO BINDING TO THE TARGET

JANOUŠEK J.,[1] BÁRTA P.,[2] TREJTNAR F.[1]

[1] Department of Pharmacology, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

[2] Department of Biophysics and Physical Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

e-mail: janousj2@faf.cuni.cz, trejtnarf@faf.cuni.cz

Ramucirumab (RAM) is a fully humanized monoclonal antibody targeted against the extracellular domain of vascular endothelial growth factor receptor 2 (VEGFR2) which serves as a key receptor of angiogenesis, including tumour angiogenesis. RAM binds to a human VEGFR2 with much greater affinity than its natural ligands and selectively inhibits its function. Several types of cancer are well known for they overexpressing of VEGFR2. Therefore, RAM with proper radiolabeling could be potentially used for diagnostic imaging or targeted radiotherapy. The aim of this work was to compare selected methods of radiolabeling in terms of binding ability to VEGFR2.

Several radiolabeling methods (direct and indirect ^{99m}Tc labeling,^{131}I direct labeling according to chloramine-T protocol and indirect ^{177}Lu labeling) were employed and the prepared radiopharmaceuticals were subsequently tested *in vitro* for receptor-ligand binding affinity with real-time radioimmunoassay. Two VEGFR2 expressing human cancer cell lines (PC3, SKOV3) were used in the binding study. A pilot *in vivo* experiment using mice bearing PC3-positive tumors was performed on the basis of *in vitro* experiment results. Regarding the ^{177}Lu labeling results we have determined radiopharmaceutical parameters following the conjugation of RAM with three selected macrocyclic chelators.

All introduced labeling methods demonstrated preserved affinity to the VEGFR2 receptor. However, both direct labeling methods augmented non-specific binding ability of RAM. Based on results from the above stated experiments, the indirectly labeled ^{99m}Tc-HYNIC-RAM was selected for the animal experiments. According to the lutetium labeling experiment results, we have selected the p-SCN-Bn-CHX-A“-DTPA chelator as the most promising agent for the further experiments in vitro and in vivo.

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

INVOLVEMENT OF FARNESOID X RECEPTOR IN NOVEL HUMAN CELLULAR MODEL OF STEATOHEPATITIS

HORVATOVA, A.[1, 2], GERBAL-CHALOIN, S.[2], PAVEK, P.[1]

[1] Department of Pharmacology and Toxicology, Faculty of Pharmacy in Hradec Kralove, Charles University, Czech Republic
[2] Institute for Regenerative Medicine and Biotherapy, INSERM U1183, University of Montpellier, France

Non-alcoholic fatty liver disease (NAFLD), the most common liver disease worldwide, encompasses liver damage initiating from simple steatosis to superimposed lobular inflammation (steatohepatitis, NASH) leading eventually to liver cirrhosis and hepatocellular carcinoma[1,2]. Up-to-date, treatment for NAFLD is limited to lifestyle modification, but recently a farnesoid X receptor (FXR) agonist, obeticholic acid, has been shown to suppress liver inflammation suggesting the importance of FXR pathway in inflammatory hepatopatologies[3]. However, currently, there is no established cellular model of human hepatocytes with hepatic immune cells with respect to NASH. Therefore, we aimed to introduce and validate human cellular model of steatohepatitis to investigate involvement of FXR pathway during this condition. For this purpose, we co-cultured hepatic HepaRG cells or primary human hepatocytes together with human macrophages differentiated from monocytic THP-1 cell line. The induced steatosis was measured by colorimetric triglyceride assay and visualized by BODIPY staining. Subsequently, we performed PCR to analyze expression of genes involved in lipid metabolism. Secreted inflammatory cytokines was determined by ELISA. Finally, expression of FXR sensitive genes was assessed by PCR. Here, we introduce a novel human *in vitro* model for steatohepatitis where we manage to induce significant steatosis in hepatocytes mimicking NAFLD without affecting cellular viability and subsequently we set up inflammatory conditions. We show that in our model, FXR pathway is inducible which allow us to investigate its involvement in NAFLD.

The study was supported by Erasmus+ programme and SVV 260414

References:

1. YOUNOSSI, Z.M., KOENIG, A.B., ABDELATIF, D., FAZEL, Y., HENRY, L., WYMER, M.: Hepatology, 2016. 64(1): p. 73-84
2. SHARMA, M., MITNALA, S., VISHNUBHOTLA, R.K., MUKHERJEE, R., REDDY, D.N., RAO, P.N.: J Clin Exp Hepatol, 2015. 5(2): p. 147-58.
3. WALLACE, K., COWIE, D.E., KONSTANTINOU, D.K., HILL, S.J., TJELLE, T.E., AXON, A., KORUTH, M., WHITE, S.A., CARLSEN, H., MANN, D.A., WRIGHT, M.C.: J Steroid Biochem Mol Biol, 2010. 120(2-3): p. 137-48.

4. ASSESSMENT OF THE STOICHIOMETRY OF IRON AND COPPER WITH DEHYDROSILYBIN A AND B

CATAPANO, M.C.,[1] VALENTOVÁ, K. ,[2] KŘEN, V. ,[2] RAWLIK T[3].,MLADĚNKA, P.[1]

[1] Department of Pharmacology and Toxicology, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

[2]Laboratory of Biotransformation, Institute of Microbiology, Academy of Sciences of the Czech Republic,

[3]University of Silesia, Faculty of Mathematics, Physics and Chemistry, Katowice, Poland

e-mail: catapanm@faf.cuni.cz

Silymarin, the standardized extract from milk thistle (Silybum marianum) is approved in the EU for supportive treatment of alcoholic liver disease. Other effects of silymarin, which is a mixture different flavolignans, on human health are suggested. The aim of this study was to analyse the interaction of pure isolated isomers of dehydrosilybin with copper and iron which can be relevant mainly in the gastrointestinal tract due to low biovailability of unmodified components of silymarin. Stoichiometry of the iron/copper complex with 2,3-dehydrosilybin A and B (DHSs) isomers was assessed using two independent methods (Job´s[1] and complementary method[2]) in four (patho)physiologically-relevant pH values (4.5. 5.5, 6.8, and 7.5). The addition of ferrous, ferric and cupric ions to dehydrosilybin at pH 5.5, 6.8 and 7.5 resulted in clear bathochromic shifts of the absorbance maxima. This confirmed the formation of complexes between metal ions and both DHSs under these conditions. On the contrary, addition of Cu^{+} ions did not modify the absorbance spectrum of DHS pointing out to inability or low affinity of this substance to chelate cuprous ions. At pH 4.5, DHS formed a complex with Cu^{2+} and Fe^{3+} ions but not with Fe^{2+}. In general under all above mentioned conditions in which the metal complex was formed, DHSs were able to chelate metal ions at two different chelation ratio (2:1 and 3:1, DHS to metal respectively). Due to different interactions of individual components of silymarin with iron and copper, the biological effect need to be traced in a more complex assay.

The study was supported by Czech Science Foundation (No. 18-00121S) and Charles University (SVV 260 414).

References

1. JOB P.: Annali di Chimica 9 1928, 113-134.
2. FILIPSKY, T.; RIHA, M.; HRDINA, R.; VAVROVA, K.; MLADENKA, P.: Bioorg. chem. 2013, 49, 1-8.

THE INFLUENCE OF FLAVONOIDS METABOLITES ON PLATELET AGGREGATION

APPLOVÁ, L.,[1] KARLÍČKOVÁ, J.,[2] TVRDÝ, V.,[1] MLADĚNKA, P.,[1]

[1] Department of Pharmacology and Toxicology, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

[2] Department of Pharmaceutical Botany, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

e-mail: applovl@faf.cuni.cz

Flavonoids seem to have beneficial effects on the cardiovascular system. These include the antiplatelet activity as well[1]. The pharmacokinetic of flavonoids is very complex and the bioavailability of parent flavonoids is minimal. Most of them are metabolized by human gastrointestinal bacteria to smaller phenolic compounds, which are subsequently absorbed into systemic circulation, where they reach higher concentrations than parent flavonoids[2]. Therefore, the contribution of these metabolites in antiplatelet effect cannot be excluded.

The available phenolic compounds were tested at different levels of platelet aggregation in whole human blood. The initial screening of 30 metabolites has shown minimal or small inhibition effect of the most tested compounds on platelet aggregation induced by arachidonic acid with the exception of four compounds. These were also able to block platelet aggregation induced by collagen. The mechanisms of action of these compounds include inhibition of platelet cyclooxygenase 1 and partly the effect on thromboxane A_2 synthase. Other potential targets of these metabolites will be investigated in the further experiments.

This study was supported by grants from the Czech Science Foundation (P303/12/G163) and Charles University (SVV 260 414).

References

1. KARLÍČKOVÁ, J., ŘÍHA, M., FILIPSKÝ, T., MACÁKOVÁ, K., HRDINA, R., MLADĚNKA, P.: Planta Med. 82 (1-2), 2016, 76-83.
2. DEL RIO, D., RODRIGUEZ-MATEOS, A., SPENCER, J. P., TOGNOLINI, M., BORGES, G., CROZIER, A.: Antioxid. Redox. Signal, 18 (14), 2013, 1818-1892.

INTERGENDER DIFFERENCES IN THE VASOACTIVE EFFECT OF SELECTED ISOFLAVONOIDS AND THE THEIR COLONIC METABOLITES.

MIGKOS, T.,[1] VOPRŠALOVÁ, M.,[1] POUROVÁ, J.,[1] NAJMANOVÁ, I.,[2] HORKÝ, P.,[3] POUR, M.,[3] MLADĚNKA, P.[1]

[1]Department of Pharmacology and Toxicology, Faculty of Pharmacy in Hradec Králové, Charles University, Hradec Králové, Czech Republic

[2]Department of Biological and Medical Sciences, Faculty of Pharmacy in Hradec Králové, Charles University, Hradec Králové, Czech Republic

[3]Department of Inorganic and Organic Chemistry Faculty of Pharmacy in Hradec Králové, Charles University, Hradec Králové, Czech Republic

e-mail: migkost@faf.cuni.cz

Isoflavonoids are well known phytoestrogens which can positively affect the human cardiovascular system[1]. Although they are poorly bioavailable after oral administration, isoflavonoids might be converted by some intestinal bacteria into absorbable metabolites[2]. Five biologically active isoflavonoids (daidzein, genistein, glycitein, biochanin A and formononetin), as well as four of their human gut metabolites (S-equol, O-desmethylangolensin, 4-ethylphenol and 2-(4-hydroxyphenyl) propionic acid) were tested in this study. Their vasodilatory action was measured *ex vivo*, on thoracic rat aorta, isolated from both male and female animals. The aortic rings were precontracted by norepinephrine (10 μM) and then the tested compounds were administered in a cumulative way, in concentrations ranging from 100 nM to 1 mM.

Dose-dependent vasodilation was evoked by most of isoflavonoids and their metabolites in biologically relevant concentrations, however their maximum effect was achieved only by relatively high doses. For some compounds a significant difference between EC_{50} values, obtained from male and female rat aorta, was observed. Interestingly, O-desmethylangolensin, an intestinal metabolite of daidzein, possessed a higher potency than its precursor on male aorta but not on the female one. We can conclude that the effect of vasoactive isoflavonoids might be influenced not only by gut metabolism but also by gender variation.

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

References

1. MIADOKOVÁ E.: Interdiscip Toxicol. 2009, 2(4):211-8.

2. SETCHELL KD, CLERICI C J.: Nutr. 2010, 140(7):1355S-62S.

INVOLVEMENT OF DRUG TRANSPORTERS IN MARAVIROC TRANSPORT ACROSS PLACENTAL BARRIER

TUPOVA L.,[1] HIRSCHMUGL B.,[2] PILAROVA V.,[3] CAPKOVA MAXOVA E.,[3] NOVAKOVA L.,[3] WADSACK C.,[2] STAUD F.,[1] CECKOVA M.[1]

[1]Department of Pharmacology and toxicology, Faculty of Pharmacy in Hradec Kralove, Charles University, Czech Republic

[2]Department of Obstetrics and Gynecology, Medical University of Graz, Austria

[3]Department of Analytical Chemistry, Faculty of Pharmacy in Hradec Kralove, Charles University, Czech Republic

e-mail: tupoval@faf.cuni.cz

The chemokine receptor 5 (CCR5) antagonist maraviroc is an HIV entry inhibitor used in combination antiretroviral therapy (cART) including prevention of mother-to-child transmission in HIV positive pregnant women. Up to date, there is a sparse data about maraviroc administration during pregnancy. Some case reports suggest limited passage of maraviroc across the placenta as indicated by the low umbilical-cord/maternal blood ratio reaching 0.33 – 0.37. This imbalance could be caused by activity of drug efflux transporter ABCB1 since maraviroc is assumed as ABCB1 substrate. However, this hypothesis has not yet been verified directly in placental tissue. Therefore, the aim of this study was to clarify the mechanisms involved in transport of maraviroc across the placental barrier.

Employing dually perfused rat term placenta, a significant asymmetry in maraviroc transplacental clearances was revealed, showing accelerated transport in the fetus-to-mother direction, when compared to the mother-to-fetus direction. This transport of maraviroc from fetal to the maternal compartment was saturable and reduced in the presence of Abcb1 inhibitor elacridar. On the other hand it did not change after addition of Abcg2 inhibitor fumitremorgin C, suggesting involvement of Abcb1- but not Abcg2- mediated efflux of maraviroc to the maternal circulation. The non-specific inhibitor ritonavir caused even more efficient reduction of maraviroc transfer to the maternal compartment indicating involvement of another transport system, except for Abcb1 in the transplacental kinetic of maraviroc.

In vitro transport assay across monolayer of human Caco-2 cell line confirmed maraviroc as a substrate of human ABCB1. Maraviroc transport in the presence of more or less specific ABCB1 inhibitors, such as elacridar, zosuquidar, verapamil, ritonavir suggested, however, an involvement of another human transporter(s) in maraviroc transfer. This hypothesis was further verified on human choriocarcinoma. BeWo cell line (clone b30), which lacks human ABCB1. Significant decrease in maraviroc accumulation into the BeWo cells was caused by polyspecific transporter inhibitors (ritonavir, verapamil and dexamethasone) indicating involvement of uptake transporter. To evaluate the interplay of ABCB1 and uptake transporters in the feto-maternal transfer of maraviroc, the perfusion study in human placental cotyledon is currently being performed and analysis processed. To conclude, maraviroc is a substrate of ABCB1. The transporter accelerates transfer of maraviroc from fetus-to-mother, nevertheless, some other transport mechanism seems to contribute to this process as well.

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.

OVERCOMING DAUNORUBICIN RESISTANCE VIA ABCC1 AND AKR1C3 INHIBITION BY CYCLIN-DEPENDENT KINASE INHIBITORS

SORF, A.[1], NOVOTNA, E.[2], HOFMAN J.[1], STAUD, F.[1], WSOL, V.[1], CECKOVA, M.,[1]

[1] Department of Pharmacology and Toxicology, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

[2] Department of Biochemical Sciences, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

e-mail: sorfal@faf.cuni.cz

Pharmacokinetic mechanisms contribute to development of multidrug resistance (MDR) to anticancer drugs. These include efflux activity of ATP-binding cassette (ABC) transporters as well as degradation of active drug forms by drug metabolizing enzymes. Daunorubicin (DNR) is an anthracycline cytostatic drug transported by various ABC pumps including ABCC1, transporter with confirmed role in MDR. It is also a well-established substrate of aldo-keto reductase (AKR) 1C3, carbonyl reducing enzyme involved in anthracycline resistance in various tumors. We investigated two cyclin-dependent kinase inhibitors, AZD5438 and R547, and their ability to modulate DNR resistance on the level of ABCC1 and AKR1C3.

We identified AZD5438, in contrast to R547, as potent inhibitor of ABCC1 enhancing significantly DNR accumulation in MDCKII-ABCC1 cells. Respecting the drugs high toxicity, resistance reversal studies were conducted. However, we observed no difference between synergism in ABCC1-expressing and parental cell line when calculated by Chou-Talalay combination index method. Both compounds were also shown to inhibit AKR1C3 in transfected HCT-116 cells. Even though less potent, only R547 exhibited synergism in AKR1C3 transfected cells compared to parental and thus revealed AKR1C3 inhibition as DNR resistance modulation option.

Even though we identified interactions of studied drugs with both ABCC1 and AKR1C3, the limited outcome of the reversal studies, taking into account inhibitory data, may be contributed to high intrinsic toxicity of both studied compounds. AZD5438 still shows potential to interact with multiple structures crucial in DNR resistance development, which could be conveniently exploited in heterogeneous resistant tumor tissue.

The study was supported by GAUK 344315/C/2015, GACR 1626849S and by SVV 2017/260-414

NOVEL RAPID BUT PRECISE SPECTROPHOTOMETRIC SCREEENING METHOD FOR DETERMINIATION OF ZINC CHELATION

TVRDY, V.,[1] CATAPANO, M.C.,[1] KARLICKOVA, J.,[2] MLADENKA, P.,[1]

[1] Department of Pharmacology and Toxicology, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

[2] Department of Pharmaceutical Botany, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

e-mail: tvrdyvac@faf.cuni.cz

Zinc is an essential and one of the most important trace element[1]. Both its lack and excess are associated with pathological states. Deficiency of zinc is more common and can also result from the treatment with approved iron/copper chelators[2]. Therefore, it was desirable to prepare a new methodology for screening of zinc chelation.

It was the aim of this work, to prepare a reliable, rapid and cheap method for the screening of zinc chelation. Spectrophotometric assessment, by use a known zinc indicator dithizone, was selected.

Initial screening performed by comparison of spectra of dithizone and its complex with zinc suggested 530 and 570 nm as suitable wavelengths for determination of zinc at pH 4.5 while 540 and 590 nm for pH 5.5-7.5. Additional research showed the lower wavelengths to be more suitable for this methodology. The sensitivity of the method was always bellow 1 μM with good linearity relationship between absorbance and zinc concentration. The method suitability was confirmed by use of two known zinc chelators, ethylenediaminetetraacetic acid (EDTA) and tetrakis (2-pyridylmethyl) ethylenediamine (TPEN). This method represents a sufficiently precise method for zinc chelation screening usable at (patho)physiologically relevant pH conditions. Such method can be employed for both screening of novel zinc chelators and for testing affinity of other metal chelators for zinc.

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

References

1. KELLEHER, S.L., MCCORMICK, N.H., VELASQUEZ, V., LOPEZ, V.: Adv. Nutr., 2(2), 2011, 101–111.
2. MACLEAN, K.H., CLEAVELAND, J.B.: Blood, 98(13), 2001, 3831–3839.

CONSTITUTIVE ANDROSTANE RECEPTOR IN THE REGULATION OF XENOBIOTIC METABOLISM

DUŠEK, J., HORVÁTOVÁ, A., ŠKODA, J., SMUTNÝ, T., PÁVEK, P.

Department of Pharmacology and Toxicology, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

e-mail: dusekja3@faf.cuni.cz

Constitutive androstane receptor (CAR) represents the superfamily of Nuclear Receptors (NR), that belong to the group of ligand-activated transcriptional factors that influence the expression of the target genes. CAR is coded by the gene NR1I3 and is primarily present in hepatocytes. This receptor belongs to the group of nuclear receptors that have no known endogenous ligands and therefore are called "orphan receptors". CAR is constitutively active and transcriptionally regulates its target genes independently on the presence of the ligands. CAR may play significant role not only in the xenobiotic metabolism but also in intermedial metabolism.[1] CAR is not adequately expressed in standard human cell lines and it quickly loses its activity in isolated human hepatocytes. The only exception is the HepaRG cell line, that expresses functional CAR after one month differentiation.[2] In this project, we have used genetically modified CAR Knockout HepaRG cell line with deleted CAR and parent HepaRG cell line, to study the role of CAR in the regulation of xenobiotic metabolism genes. We have focused on the expression of two most important phase I enzymes, CYP3A4 and CYP2B6, that are controlled mainly by PXR or CAR, respectively. Based on our data, CAR Knockout HepaRG cell line seems to be functional and potential good model for the study of CAR-mediated regulation.

The study was supported by GAUK 170/50/75014

References

1. DI MASI, A., DE MARINIS, E., ASCENZI, P., MARINO, M.,.: Mol Aspects Med., 30(5), 2009, 297-343.
2. LI, D., MACKOWIAK, B., BRAYMAN, TG., MITCHELL, M., ZHANG, L., HUANG, SM., WANG, H.: Biochem Pharmacol., 1;98, 2015, 190-202.

ASPECTS OF POST-TRANSCRIPTIONAL REGULATION OF PREGNANE X RECEPTOR

SMUTNÝ, T., PÁVEK, P.

Department of Pharmacology and Toxicology, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

e-mail: smutt6aa@faf.cuni.cz

Pregnane X receptor (PXR) is an important transcription factor playing a critical role in regulation of xenobiotic and endobiotic metabolism. Although PXR was broadly studied regarding its function, less is known about mechanisms controlling its own expression. MiRNAs are short (~22 nt) nucleotides, which target responsive elements (MREs) predominantly within 3´-untranslated region (3´-UTR) of mRNA leading to suppression of gene expression. Up to date, only several MREs were described within 3´-UTR of PXR spanning 1272 nt. In our study, we tried to uncover mechanisms standing behind post-transcriptional regulation of PXR.

At first, gene reporter study revealed a strong suppressive role of 3´-UTR of PXR in hepatoblastoma-derived HepG2 cells. Since miR-18a-5p responsive element was predicted *in silico* and recently confirmed within 3´-UTR of PXR, we tested whether miR-18a-5p could be responsible for inhibition of PXR expression. As shown by RT-qPCR, miR-18a-5p is substantially expressed in HepG2 cells. However, activity of reporter vector including MRE did not differ from that of control vector comprising reverse sequence of MRE. In the same line, antisense oligonucleotide against miR-18a-5p did not lead to increased activity neither MRE or 3´-UTR PXR vectors. Forced expression of miR-18a-5p caused decrease in activity of both MRE or 3´UTR PXR vectors suggesting that MRE for miR-18a-5p is functional.

In conclusion, 3´-UTR of PXR appears to contribute to regulation of PXR expression. More other aspects e.g. feedback regulation of PXR will be discussed during presentation.

INTERACTIONS OF ANTIRETROVIRALS WITH DRUG TRANSPORTERS; ROLE IN THE PHARMACOKINETICS

REZNICEK, J., CECKOVA, M., STAUD, F.

Department of Pharmacology and Toxicology, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

e-mail: reznj7ba@faf.cuni.cz

The efficacy of anti-HIV therapy depends strongly on maintaining sufficient levels of antiretroviral drugs in plasma and body tissues and on the ability to prevent the development of resistant viral strains. Co-administration of two or three antiretrovirals, so called combination antiretroviral therapy (cART), is therefore recommended in most therapeutic regimens, however, it bears the risk of drug-drug interactions (DDI). In particular, DDI on membrane drug transporters can significantly affect absorption, distribution and elimination of co-administered therapeutic compounds. Here we aimed to study interactions of several antiretrovirals with selected ABC and SLC drug transporters using *in vitro* accumulation and transport assays on different cell lines expressing selected human ATP-dependent (ABC) transporters or solute carriers (SLC transporters), *in situ* method of dually perfused rat placenta or *in vivo* pharmacokinetic study on Wistar male rats. We revealed emtricitabine as substrate of MATE1 but not OCT1, OCT2, P-gp, BCRP or MRP2 membrane transporters. Further, we proved that etravirine is inhibitor of BCRP but not P-gp and is able to increase transport of tenofovir disoproxil fumarate (TDF) across placenta from mother to foetus. We also described rilpivirine as an inhibitor of P-gp and BCRP but not MRP2, OCT1, OCT2 or MATE1 and its ability to enhance bioavailability of perorally administered abacavir. We further discovered, that efavirenz is inhibitor of OCT1, OCT2, MATE1 and MRP2 but is not a substrate of P-gp, BCRP or MRP2. Its DDI on OCT and MATE1 transporters significantly decreases renal clearance of lamivudine, prolongs its elimination half-life and leads to increased lamivudine accumulation in renal tissue. Our results also show, that lamivudine transplacental passage is probably not influenced by P-gp, MRP2 nor BCRP, but it seems to be influenced by MATE1. In conclusion, our results clearly show high potential of several antiretrovirals to cause transporter-mediated DDI. These data should help optimize the therapy of HIV positive patients

The study was supported by the Grant Agency of the Charles University in Prague (SVV/2017/260 414).

Clinical and Social Pharmacy

COLD, BUT RISING STAR OF VALUE ADDED MEDICINES IN EUROPE

RÖDER, J., DOHNAL, F., KOSTŘIBA, J.

Department of Social and Clinical Pharmacy, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

e-mail: rodej6aa@faf.cuni.cz

Drug therapy today is moving from a one-fits-all patients approach towards drug therapies in conjunction with disease specific diagnostics to ‘‘personalized medicines’’ as well as patient centric therapies for different patient populations towards ‘‘individualized medicines’’.[1] The current pool of existing molecules potentially re-positioned, re-formulated or combined with new technological platforms and services might offer therapeutic alternatives and opportunities for patients and healthcare systems. Even if this concept has been known for many years and despite their significance, refined pharmaceuticals are still described in a rather confusing manner.[2] There is still no agreed terminology to name properly the pool of those re-innovated products, although Medicines for Europe[3] established one single terminology known as Value Added Medicines.

Comparing utilization of such an innovation, significant difference has been spotted between US and EU. Based on selected case studies (e.g. Risperidone thin film), it is perceived, that the bottleneck in better utilization of such a concept seems to be market access and non-harmonized pricing conditions in the various European countries, thus preventing its broader use.

References

1. STEGEMANN, S., KLEBOVICH, I., ANTAL, I., BLUME, H., MAGYAR, K., NÉMETH, G., PAÁL, T., STUMPTNER, W., THALER, G., VAN DE PUTTE, A., SHAH, V.: European Journal of Pharmaceutical Sciences, 44, 2011, 447–454
2. (Barei 2016)
3. http://www.medicinesforeurope.com/value-added-medicines/

THE BIOIMPEDANCE SPECTROSCOPY APPLICATION IN PREGNANT WOMEN WITH PRETERM PREMATURE RUPTURE OF MEMBRANES

JOSKOVA V.,[1] PATKOVA A.,[1] GREGOR M.,[2] KACEROVSKY M.,[2] HRONEK M.[1]

[1] Department of Biological and Medical Sciences, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

[2] Department of Obsterics and Gynecology, University Hospital, Hradec Králové, Czech Republic

e-mail: joskovv@faf.cuni.cz

Many pregnancies are complicated by preterm premature rupture of membranes (pPROM) that causes approximately one third of preterm deliveries. It can lead to significant perinatal morbidity and risk of fetal death. The most patients deliver within one day of pPROM, whereas especially patients in lower gestation week give birth within 1 to 4 weeks. Unfortunately, a tool that could determine the term of delivery appropriately is still lacking. During pregnancy, there is an increase in the volume of maternal body fluids, which culminates before labor. These changes are measurable by bioimpedance spectroscopy (BIS). Therefore, the hypothesis of this study was to evaluate the BIS application for delivery term prediction. In this study, 97 pregnant women after pPROM were examined by BIS on the day of diagnosis and compared with 173 examinations of healthy pregnant women in different period during pregnancy including the delivery day. Our results show that during pregnancy together with increasing total body fluids, the resistance measured by BIS decreases with the lowest value on the delivery day. No significant difference between this value in healthy laboring women and laboring women after pPROM ($p = 0.920$) was demonstrated. What is important, resistance is significantly different from mother with pPROM who is giving child and from pregnant woman delivering ≥ 7 days of diagnosis ($p < 0.0001$). Confirmation of this finding gives this simple method for the term delivery prediction that could improve care of pregnant women with pPROM.

The study was supported by the Faculty of Pharmacy SVV/2017/260417, Charles University in Prague GA UK 772216, MH CZ - DRO (UHHK, 00179906), PROGRES Q42.

ADJUSTMENTS IN ENERGY EXPENDITURE AND SUBSTRATE OXIDATION DURING PREGNANCY

PATKOVA, A.,[1,2] JOSKOVA, V.,[1,2] ZADAK, Z.,[2] KACEROVSKY, M.,[3] HRONEK, M.,[1,2]

[1] Department of Biological and Medical Sciences, Faculty of Pharmacy in Hradec Kralove, Charles University, Czech Republic

[2] Department of Research and Development, University Hospital Hradec Kralove, Czech Republic

[3] Department of Obstetrics and Gynecology, University Hospital Hradec Kralove, Czech Republic

e-mail: patkova2@faf.cuni.cz

Metabolic adjustments occur during pregnancy to support fetal growth; however, there are only a few longitudinal studies describing these changes over the time of pregnancy. By indirect calorimetry (IC), we measured resting energy expenditure (REE) and substrate oxidation in healthy, active, non-smoking, pregnant women after 12 h of fasting in 3 different periods – 2nd trimester (23.39 ± 2.16 weeks of gestation); 3rd trimester (31.04 ± 1.13 weeks of gestation) and late 3rd trimester close to delivery (37.41 ± 0.72 weeks of gestation). With increasing gestational age, O_2 consumption (r = 0.364; $p = 3.000 \times 10^{-5}$), CO_2 production (r = 0.463; $p = 4.695 \times 10^{-8}$), respiratory quotient (r = 0.249; p = 0.005), REE (r = 0.402; $p = 3.000 \times 10^{-6}$) and carbohydrate oxidation (r = 0.249; p = 0.005) were increasing. Only protein oxidation was decreasing (r = -0.222; p = 0.013). Very similar correlations were found between all mentioned parameters and the number of days from examination until delivery. What is more, protein oxidation in the third period inversely correlated with the newborns birth weight (r = -0.464; p = 0.019). These results indicate increasing REE and higher use of carbohydrates as a source of energy with increasing length of pregnancy caused probably by the alteration in maternal tissue and metabolism to ensure foetal growth and development. Protein utilization is conversely decreased to retain protein for tissue synthesis. Metabolic examination done by IC during pregnancy could be potentially useful since it allows recommendations on energy and macronutrients for achieving a balanced intake.

The study was supported by GA UK č. 772216, SVV/2017/260417, MH CZ – DRO (UHHK, 00179906) and PROGRES Q42.

POTENTIALLY INAPPROPRIATE MEDICATION USE IN NURSING HOME RESIDENTS IN THE CZECH REPUBLIC: RESULTS FROM THE EU SHELTER PROJECT

PŘÍHODOVÁ V. [1], GREŠÁKOVÁ S.[1], FIALOVÁ D. [1,2]

[1] Department of Social and Clinical Pharmacy, Faculty of Pharmacy in Hradec Králové, Charles University
[2] Department of Geriatrics and Gerontology, 1st Faculty of Medicine, Prague, Czech Republic
Email: gresakova.silvia@seznam.cz

Introduction: Potentially inappropriate medication use (PIM use) presents a frequent problem of potentially inappropriate prescribing that may lead to higher risk of adverse drug events in older patients, often suffering from multiple disorders, polypharmacy and presenting higher degree of frailty. Analyses of the EU SHELTER project (Services and Health in the Elderly in Long-Term care, 7th FP, 2009-2014) focused on description of comprehensive clinical characteristics and prescribing practices in 4156 long term care residents in 7 EU countries (Czech Republic, Italy, Germany, Netherlands, Finland, UK, France) and Israel. This work presents findings of PIM prescribing in nursing home residents in the Czech Republic after application of Beers 2012 criteria, Czech national 2012 consensus on PIMs and STOPP/START criteria.

Methods: 490 nursing home older patients (65+) residing in 10 Czech long-term care facilities in geographically different areas were prospectively assessed during the baseline period of the EU SHELTER project. In this prospective assessment, RAI-LTCF comprehensive geriatric tool was applied including different characteristics (e.g. demographic characteristics, functional status and mobility, clinical characteristics and medication use) and various functional scales. Prevalence of PIM use was determined using 3 sets of standard explicit criteria: Beers 2012 criteria, Czech national 2012 consensus on PIMs and STOPP/START criteria. Descriptive statistical methods were applied using SPSS Software vers. 12.

Results: The highest prevalence of potentially inappropriate medication use (62,3%) was determined by Czech national consensus, then by Beers 2012 criteria (60,2%) and STOPP/START criteria (44,5%/52,9%). The most prevalent prescribing problems according to

Czech national consensus were: long-term use of benzodiazepines (BZDs) in depressive patients (7,8% in the total sample), untreated constipation caused by opioid analgesics (7,4%), long-term use of NSAIDs, indication of ACE-I without clinical monitoring (6,1%), use of verapamil in patients with chronic constipation (3,9%) and use of doxazosin in older patients having urinary incontinence (2,9%). The most prevalent problems according to Beers 2012 criteria were: long-term use of BZDs in patients with the history of falls (6,3%) and in cognitively impaired older residents (4,3%), long-term use of zolpidem in cognitively impaired patients (4,3%) and long-term use of ASA or clopidogrel and NSAIDs without gastroprotection (3,7%). Among problems of undertreatment according to START criteria were identified the most frequently: no anticoagulation treatment in atrial fibrillation (7,1%), no ACE-I or sartanes in patients suffering from chronic heart failure (4,5%) and no antidepressive treatment in patients having diagnosed depression (3,9%).

Conclusion: Application of Czech national consensus of PIMs yielded the highest prevalence of prescribing problems compared to Beers 2012 and STOPP/START criteria in Czech nursing home residents. The Czech national consensus is a specific tool summarized from different, until now published explicit criteria, including also Beers and STOPP/START criteria. This tool reflects specific availability of PIMs on the Czech pharmaceutical market and national prescribing habits.

References:

1. The American Geriatrics Society 2012 Beers Criteria Update Panel. American Geriatrics Society updated Beers criteria for potentially inappropriate medication use in older adults. J Am Geriatr Soc 2012; 60(4): 616-631.
2. GALLAGER P., RYAN C., BYRNE S., KENNEDY J., O´MAHONY D. STOPP (Screening Tool of Older Person´s Prescriptions) and START (Screening Tool to Alert doctors to Right Treatment). Consensus validation. Int J Clin Pharmacol Ther 2008; 46(2): 72-83.
3. FIALOVÁ D, TOPINKOVÁ E, BALLÓKOVÁ A, MATĚJOVSKÁ KUBEŠOVÁ H. Expertní konsensus ČR 2012 v oblasti léčiv a lékových postupů potenciálně nevhodných ve stáří. Oddíl I. Vhodnost volby léčiv a dávkovacích schémat u geriatrických pacientů, Oddíl II. Interakce lék-nemoc ve stáří. Klin Farmakol Farm 2013; 27(1): 18-28.

DRUG UTILIZATION STUDY OF ANTICOAGULANTS

DVOŘÁČKOVÁ, S.,[1] ZIMČÍKOVÁ, E.,[1] MALÁ-LÁDOVÁ, K.,[1] MALÝ, J.,[1] PENKA, M.,[2] KOLÁŘ, J.,[1]

[1] Department of Social and Clinical Pharmacy, Faculty of Pharmacy in Hradec Králové, Charles University

[2] Department of Clinical Hematology, The University Hospital Brno

e-mail: dvoracsi@faf.cuni.cz

Anticoagulants are used in the prevention and treatment of thromboembolic events. Mainly warfarin had been used in the area of oral anticoagulants (OACs) until the direct oral anticoagulants (DOACs) were approved, therefore some changes in OACs utilization are expectable. The aim of the study was to explore drug utilization patterns of anticoagulants in the Czech Republic (CR) during the period 2007 – 2016. A retrospective analysis was conducted using the data from the State Institute of Drug Control database. This database contains reports of drug supplies from distributors to pharmacies, health care facilities, vendors of selected pharmaceuticals, and veterinarians. All anticoagulants, approved in the CR during the study period, were included in the study. Drug utilization was calculated as a ratio of number of defined daily doses per thousands of inhabitants per day (DDD/TID). Also the anticoagulants expenditures analysis was performed. Descriptive statistical analysis was provided to describe drug utilization patterns. Cross-correlation was applied to assess NOACs and warfarin utilisation relationship. The utilization of all anticoagulants had increased during 2007 – 2016 from 14.15 DDD/TID to 26.42 DDD/TID. The DOACs utilization increased from 0.002 DDD/TID in 2008 to 5.26 DDD/TID in 2016. On the contrary, warfarin utilization decreased after DOACs approval from 10.03 DDD/TID in 2007 to 8.36 DDD/TID in 2008, however, its current utilization almost stagnates. Increase in parenteral anticoagulants utilization was also apparent at low molecular weight heparins, nevertheless use of unfractionated heparin and fondaparinux was low. The financial expenditures analysis revealed that the OACs expenditures increased obviously, due to the higher cost of DOACs. In conclusion, the results showed increasing utilization of anticoagulants and that increasing use of warfarin was stopped by rising use of DOACs significantly. This trend, also notified in other studies, can contribute to further research and enhance anticoagulants practical use manners.

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

ANALYSIS OF FACTORS INFLUENCING THE RISK OF FALLS – PILOT RESULTS

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]

[1]Department of Social and Clinical pharmacy, Pharmaceutical faculty in Hradec Králové

[2]Department of Nursing, Midwifery and Emergency Care, Faculty of Health and Social Sciences, University of South Bohemia in České Budějovice

Email: vosatkaj@faf.cuni.cz

By hospitalized patients, the risk of falling is increased due to the changes of environmental factors and very often by modification or changes in pharmacotherapy. The goal of the project was to analyze the impact of pharmacotherapy and other factors associated with falls in patients who fell during the first 6 months of 2017 within their hospitalization in 16 selected departments of South Bohemia Hospitals.

The results of this prospective case-control study were gained from a web application containing data about patients who fell down. The obtained data originated from patient´s medical documentation (e.g. drug and personal anamnesis, selected laboratory results) and were completed with other information (e.g. associated risk factors etc.). Each fall was matched with 10 control patients who did not fall during hospitalization and had similarities in some defined parameters (hospital department, sex, age, duration of hospitalization, number of drugs). The analysis was mainly aimed to identify risk drugs, risk diagnosis and other risk factors, which could have led to falls. The analysis of risk diagnosis was conducted by literature review and it considered 30 risk diagnoses. Potential and individual risks were determined for each patient who fell down. The potential risk represented all drugs that showed an increased risk of falls described in current literature or it was possible to assume risk according to mechanism of action. If a clinical pharmacist could not exclude drug influence on fall probability then the drug was marked as the individual risk. The overall influence of pharmacotherapy on falls was classified by the Likert scale. The obtained pilot results were described by methods of descriptive statistics.

157 patients were analysed with the representation of 52% men and the median age reached 79 ± 12.2 years old. Total number of drugs was 1271 for all patients and the potential

risk was reported in 45% of them. Clinical pharmacist marked the individual risk in 42% of all potential risk drugs. The average number of diagnoses with a fall risk was 2.7 per patient.

The influence of administered drugs on the chance of fall was detected in our study. One of the elimination ways is to engage the clinical pharmacist into the multidisciplinary team that takes care of patients in health-care facilities.

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.

POTENTIALLY INAPPROPRIATE MEDICATION USE („PIM“ USE) IN OLDER PATIENTS IN A TERTIARY CARE TEACHING HOSPITAL IN SOUTH INDIA: PREVALENCE AND RISK FACTORS

BANDARI, D,K* [1,2].; FIALOVA, D. [1]; BHAGAVATHULA, A, S[3]

[1]Social and Clinical Pharmacy, Faculty of Pharmacy, Charles University, Czech Republic.

[2]Clinical Pharmacy, Vaagdevi College of Pharmacy, Warangal, Telangana, India.

[3]Clinical Pharmacy, University of Gondar College of Medicine and Health Sciences, Gondar, Ethiopia.

Email: deepakkb@faf.cuni.cz, laxmideepak.pharma@gmail.com

Background and Objective: Even if the quality and safety of drug prescribing in older population is a major global health care concern, Potentially Inappropriate Medications (PIMs) are still widely prescribed in older adults both outside the hospitals as well as in acute care. Strategies supporting the medication safety in older population emphasize nowadays to avoid unnecessary use of PIMs because of frequent drug-related problems. Older adults tend to use multiple drugs and frequent age-related physiological changes, multiple disorders and clinical problems also contribute to potential inappropriateness of some medications. The objectives of this study were to determine the prevalence and risk factors of PIM use in older patients admitted to a tertiary care teaching hospital in Warangal, India and to describe the most frequently documented PIMs on different acute care wards.

Setting and Method: A prospective observational study was carried out among elderly patients admitted to a 1000 bedded tertiary care teaching hospital, Warangal, South India from November 2016 to June 2017. Data on age, gender, diagnoses, duration of hospital stay, treatment and therapeutic outcomes were collected. Prescriptions were assessed using potentially inappropriate medications defined by American Geriatric Society 2012 Beer‘s criteria. Patients were counselled regarding disease conditions and medication use. Data has been analysed using SPSS.

Main outcome measures: The prevalence of PIM use (total prevalence on admission and differences between clinical wards) and risk factors have been analyzed in older patients admitted to acute care.

Results: A total of 1050 geriatric patients (75.04% males and 24.96% females) were admitted to acute terciary care hospital in India during the study period. The average age of geriatric patients was 71.69 ± 4 years. The prevalence of Polypharmacy (measured as 5 and more medications) was 74% and users of PIMs were mostly admitted to Cardiology (38%), Department of Internal Medicine (24%) and Surgery (12%). According to the Beers 2012 Criteria, a total of 798 admitted patients (76%) were prescribed at least one PIM. Out of all the medications used, 39.5% were PIMs. In 25.8% of older patients these PIMs should have been avoided independently of disease conditions, in 6.1% were inappropriate in the presence of certain illnesses or symptoms and in 4.6% PIM users could be prescribed with a special caution. In the multivariate regressive analytical model; variables of Polypharmacy ($p=0.0187$), Psychiatric disorders ($p \leq 0.0001$) and Cerebrovascular diseases ($p=0.0036$) were significantly associated with PIM use.

Conclusion: Usage of PIMs is highly prevalent in older patients, particularly outside the hospitals. The associated factors of PIM use in acutely admitted older adults were mainly polypharmacy and psychiatric or cerebrovascular morbidity. Health care professionals outside hospitals are nowadays recommended to follow geriatric guidelines in order to significantly reduce unnecessary and risky use of PIMs. These professionals are requested to intervene through proper knowledge in order to reduce frequent adverse drug events and high hospitalisation rates in geriatric population. In this effort, clinical pharmacy services and interdisciplinary cooperation with clinical pharmacists are very important.

References:

1) BEERS MH, OUSLANDER JG, ROLLINGHER I, REUBEN DB, BROOKS J, BECK JC: Explicit criteria for determining inappropriate medication use in nursing home residents. UCLA division of geriatric medicine. Arch Intern Med 1991, 151:1825–1832.
2) BEERS MH: Explicit criteria for determining potentially inappropriate medication use by the elderly: an update. Arch Intern Med 1997, 157:1531–1536.
3) FIALOVÁ D, TOPINKOVÁ E, GAMBASSI G, FINNE-SOVERI H, JÓNSSON PV, CARPENTER I, SCHROLL M, ONDER G, SØRBYE LW, WAGNER C, REISSIGOVÁ J, BERNABEI R, AdHOC Project Research Group FT. Potentially Inappropriate Medication Use Among Elderly Home Care Patients in Europe. *JAMA.* 2005;293(11):1348–1358.

KNOWLEDGE OF DIFFERENT PHARMACY PROGRAM STUDENTS ON RATIONAL GERIATRIC PHARMACOTHERAPY

BHAGAVATHULA, A, S[1,;] BANDARI, D, K* [2,3].; FIALOVA, D. [2];

[1]Department of Clinical Pharmacy, University of Gondar College of Medicine and Health Sciences, Gondar, Ethiopia

[2]Department of Clinical and Social Pharmacy, Faculty of Pharmacy, Charles University, Czech Republic

[3]Department of Clinical Pharmacy, Vaagdevi College of Pharmacy, Warangal, Telangana, India

Email: bhagavaa@faf.cuni.cz, akshaypharmd@gmail.com

Introduction: Knowledge of rational geriatric pharmacotherapy and appropriate geriatric care becomes an important issue with aging of the world population and increasing proportion of geriatric patients. The aim of the study was to assess and compare the level of knowledge in rational geriatric pharmacotherapy and geriatric care among students of different pharmacy programs in Telengana, India.

Methods: A multicentre, cross-sectional design was used to collect the data for this study. The study was conducted between 1st of November and 31st December 2014 in the state Telangana, India. Self-administered questionnaire was used to collect the data by using a convenient sampling technique from the final-year students of three different pharmacy programs enrolled at four colleges of pharmacy in Telangana state, India (number of responders were 438 out of 720 students). A 31 item open-ended questionnaire containing questions related to sociodemographic features (7 items), and remaining 24 questions related to geriatric care: aging (5 items), physical activity (6 items), pharmacotherapy (10 items) and nutrition (3 items). The geriatric pharmacotherapy knowledge scores ranging from 1-25.

Results: A total of 438 pharmacy students with different pharmacy degree levels (109 diploma in pharmacy students, 198 bachelor of pharmacy students, and 131 Doctor of pharmacy students) completed the survey. The overall mean score of knowledge of geriatric issues was 7.99 ± 2.96 among pharmacy students. The PharmD students recorded significantly the highest mean score of knowledge (Mean score = 8.88) than the D.Pharm and B.Pharm students (7.55 and 7.72, respectively; $P < 0.001$).

Conclusions: The level of knowledge related to rational geriatric pharmacotherapy and geriatric care was low among pharmacy students in India. Significant differences were founded among the 3 pharmacy program students. An optimal plan of education should be established to begin more geriatric-focused courses and training in future pharmacy curriculum in order to enhance the knowledge of pharmacy students and their preparedness for their clinical practice after graduation.

References:

1. SRIKANTH BA, REDDY KR, JAIN SK. Geriatric Care improving adherence Through Pharmacy Practice. International Pharmacy Journal. 2012;28(1):46-9.

3. MARGARET JANE MOORE, PHYLLIS MOIR, MICHELE M. PATRICK, JEANNE ALONGI, BONITA, LYNN BEATTIE, LAURENCE G. BRANCH ET AL. The State of Aging and Health in America. Washington: DC: Merck Institute of Aging & Health2004.

4. WORLD HEALTH ORGANIZATION. World Health Day 2012: ageing and health: toolkit for event organizers. Geneva: World Health Organization2012.

5. GOPAL K INGLE, ANITA NATH. Geriatric health in India: Concerns and solutions. Indian Journal of Community Medicine. 2008;33(4):214-8. doi:10.4103/0970-0218.43.

CARDIOVASCULAR COMORBIDITIES AND RISK FACTORS IN ACCORDANCE WITH PHARMACOGENETIC OF METHOTREXATE IN RHEUMATOID ARTHRITIS PATIENTS – PRELIMINARY DATA

HLOCH, K.,[1] DOSEDĚL, M., [1] SOUKUP, T.,[3] ŽALOUDKOVÁ, L.,[4] PÁVEK, P., [2]

[1] Department of Social and Clinical Pharmacy, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

[2] Department of Pharmacology and Toxicology, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

[3] Division Rheumatology, 2nd Department of Internal Medicine-Gastroenterology, Faculty of Medicine and University Hospital in Hradec Kralove, [1]Charles University, Czech Republic

4 Department of Clinical Biochemistry, University Hospital Hradec Králové

e-mail: hlochk@faf.cuni.cz

Patients with rheumatoid arthritis (RA) have a higher occurrence of cardiovascular risk factors and thus cardiovascular diseases (CVD) are by about 50% often than in the general population. Despite modern approach in RA therapy, methotrexate (MTX) is still considered as an anchor drug. Due to its anti-inflammatory activity, MTX works favorably on the risk of CVD development.

The aim of our study is to determine whether folate pathway related single nucleotide polymorphisms (rs2298383, rs3761422, rs2267076, rs2236624, rs17602729, rs2372536, rs1127354, rs2236225, rs1801131, rs1801133, rs4149056) might be predictive of increased cardiovascular comorbidities in RA patients treated with oral MTX.

Data from genotyped patients were collected in the University Hospital in Hradec Králové from the 1st of September2016 to the 31st of May 2017. Personal and drug anamnesis were obtained from medical documentation. Moreover, other data such as blood pressure, height, weight, waist circumference and EKG were collected. Blood and urine samples for biochemical and hematological analysis were also gathered during patients visit.

115 patients were enrolled (34 men and 81 women) with 24 CVD and 219 cardiovascular risk factors in total. The average age was 60.6 ± 11.8. ΔDAS reflecting the change in RA disease activity was 2.07 and the average number of drugs used by these patients was 7.8.

Treatment with MTX is connected with many adverse reactions, which could lead to serious side effects. On the other hand, in some patients, the usual dose of MTX is without therapeutic effect. We suppose that these situations might be caused by different genetic predisposition in the genes of the folate pathway. If we find a correlation between higher prevalence of CVD and those SNPs it would help us to individualize dosing depending of patient´s genotype.

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

PREVALENCE OF DIETARY SUPPLEMENT USE IN PATIENTS IN PRE-OPERATIVE PERIOD

STRAZNICKA, J.,[1] FALTYSOVA, A.,[1] POKLADNIKOVA, J.,[1] JAHODAR, L.,[2]

[1] Department of Social and Clinical Pharmacy, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

[2] Department of Pharmaceutical Botany and Ecology, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

e-mail: straj7aa@faf.cuni.cz

The prevalence of complementary and alternative medicine (CAM) use by presurgical patients ranges from 7.2 % to 49.8 %.[1, 2] Use of some dietary supplements (DS), mostly herbal remedies, may lead to an increase of adverse drug effects during or after the surgery.[3] In the Czech Republic, the prevalence of vitamins/minerals and herbal remedies use is 54.6 % and 47.8 % in the general population, respectively.[4] No data on DS use in presurgical patients exists therefore the aim of this study was to determine the prevalence rate of DS use in presurgical patients. A self-administered questionnaire was distributed among 180 presurgical patients at nine hospital departments at the University Hospital Hradec Kralove from July 2017 to January 2018. A total of 108 respondents participated in the study (response rate 60 %). Fifty five percent of respondents used some form of CAM less than 30 days before surgery, 89.8 % of these respondents used at least one DS. The most commonly used DS were herbals (64.4 %), non-herbal DS (49.2 %) and vitamins and minerals (37.3 %). A total of 31 respondents consumed herbs with a daily diet. There is a relatively high prevalence rate of DS use in presurgical patients. Further analysis is needed to identify potential risks associated with DS use prior surgery as well as patients' awareness of such risks.

The study was supported by SVV 260 417.

References

1. LUCENTEFORTE, E., GALLO, E., PUGI, A., et al.: Evidence-Based Complementary and Alternative Medicine, 2012, 2012, 1-6.
2. SOÓS, S. A., JESZENÖI, N., DARVAS, K., HARSÁNYI, L.: BMC Complementary and Alternative Medicine, 15, 2015, 358.
3. deAZEVEDO PRIBITKIN, E.: Seminars in Integrative Medicine, 3(1), 2005, 17-23.
4. POKLADNIKOVA, J., SELKE-KRULICHOVA, I.: Research in Complementary medicine, 23(1), 2016, 22-28.