ABSTRACTS

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PHARMACEUTICAL ANALYSIS SECTION

DETERMINATION OF ARTIFICIAL COLORANTS BY SEQUENTIAL INJECTION CHROMATOGRAPHY USING MODERN MONOLITHIC COLUMNS

DAVLETBAEVA, P. N.,[1] CHOCHOLOUŠ, P.,[2] SOLICH, P.,[2] BULATOV, A. V.[1]

[1] Department of Analytical Chemistry, Institute of Chemistry, Saint Petersburg, Saint Petersburg State University, Russian Federation
[2] Department of Analytical Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
e-mail: poli1993@yandex.ru

This work is dedicated to development and optimization of separation and determination of three water soluble food colorants (Sunset Yellow FCF, Carmoisine, Green S) by sequential injection chromatography (SIC) with spectrophotometric detection with wavelenghts 480, 516 and 630 nm respectively. In the research monolithic columns Chromolith® CN 50-4.6 and Chromolith® Diol 50-4.6 were tested. Mobile phases were based on mixture of water and ammonium acetate. Gradient elution was involved and achieved by automated reproducible mixing of water and ammonium acetate buffer solution in the holding coil of the SIC system. Gradient profile, flow rate and volume of mobile phase were optimized using each column for rapid separation with good resolution of analytes. Analytical characteristics will be presented.

The technique was successfully applied to the separation of colorants in Coldrex Max Grip Lesní Ovoce as a sample. The quantification was performed by standard addition method.

The study was supported by International Visegrad Fund.

QUALITY CONTROL OF FOOD SUPPLEMENTS AVAILABLE ON THE CZECH MARKET USING CORE-SHELL COLUMN CHROMATOGRAPHY

FIBIGR, J., ŠATÍNSKÝ, D.

Department of Analytical Chemistry, Faculty of Pharmacy in Hradec Králové,
Charles University, Czech Republic
e-mail: fibigrja@faf.cuni.cz

Many biologically active substances started to be used in commercial preparations, as food supplements. However no analytical methods have been proposed for quality control of nutraceuticals with these substances, such as resveratrol and indol-3-carbinol yet.

In our first study[1] a new HPLC method using fused-core column for fast separation of resveratrol and its glycosylated form polydatin has been developed. Both forms show cardioprotective properties, which are associated with their anti-inflammatory abilities and scavenging of reactive oxygen species. The optimal separation conditions for resveratrol, polydatin and internal standard *p*-nitrophenol were found on the fused-core column Ascentis Express ES-Cyano (100 × 3.0 mm), particle size 2.7 µm, with mobile phase acetonitrile/0.5% acetic acid pH 3 (20:80, v/v) at a flow rate of 1.0 mL min^{-1} and at 60 °C.

In the second study[2] a new HPLC method using core-shell column for separation of indole-3-carbinol and its condensation/degradation products was developed and used for quantitative determination of indole-3-carbinol in nutraceuticals. Indole-3-carbinol is natural glucosinolate identified for prevention of human breast, prostate and other types of cancer. Separation of indole-3-carbinol and internal standard ethylparaben was performed on the core-shell column Kinetex 5µ XB-C18 100A (100 × 4.6 mm), particle size 5.0 µm, with mobile phase acetonitrile/water according to the gradient program at a flow rate of 1.25 mL min^{-1} and at temperature 50 °C.

The both developed methods provided rapid and accurate tool for quality control of nutraceuticals based on extracts with mentioned compounds content.

The study was supported by the project of specific research no. SVV 260 184.

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DEVELOPMENT OF HPLC-FD METHOD FOR DETERMINATION OF ARGININE AND ITS METABOLITES IN CHRONIC WOUND FLUIDS

JAVORSKÁ, L.,[1,2] AUFARTOVÁ, J.,[1,2] PISKÁČKOVÁ, H.,[1] 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] 3rd Internal Gerontometabolic Clinic, University Hospital Hradec Králové, Czech Republic
e-mail: javorskl@faf.cuni.cz

Patients with chronic wounds present a serious problem in health care. Prolonged hospitalization creates high costs and impairs the well-being of the patient. Wound healing is a complex and dynamic process affected by many factors. It was discovered that important role in a wound healing process plays nitric oxide (NO) pathway.[1] Amino acid arginine is the sole precursor of nitric oxide and its level is variable during wound healing.[1] Monitoring of arginine metabolism directly in the wound liquid, could be another indicator of chronic wound healing process and significantly improve therapy of non healing wounds.

The aim of this project was to developed HPLC method with fluorescence detection for the determination of arginine, ornithine and citrulline in a fluid from non healing wounds. Separation of these analytes was performed using C18 monolithic column. Sodium acetate buffer (solution A) and mixture of ACN and MeOH (solution B) were used as the mobile phase. Gradient elution mode was applied. Total time of analysis (including a column-wash step) was 14 min. The method was validated by testing its linearity, precision, accuracy, recovery, robustness and detection/quantitation limit values. The method was linear over the range of 2.87–43.05 μmol/L for arginine, 2.97–44.45 μmol/L for ornithine, 2.85–42.81 μmol/L for citrulline and exhibited good correlation coefficient higher than 0.999. This method will be used for clinical practice in the Research laboratory of 3rd Internal Gerontometabolic Clinic in University Hospital Hradec Králové.

The study was supported by the SVV 260 184, Project MH CZ-DRO (UHHK, 00179906), PRVOUK P37/12.

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DEVELOPMENT OF EXTRACTION PROCEDURE FOR THE DETERMINATION OF THIAMINE AND ITS DERIVATIVES IN BIOLOGICAL FLUIDS

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] 3rd Internal Gerontometabolic Clinic, University Hospital in Hradec Králové, Czech Republic
e-mail: jencoj@faf.cuni.cz

Thiamine, also called vitamin B_1, belongs to a broad group of water-soluble vitamins. All forms of vitamin B_1 are important precursors involved in cellular energy metabolism and proper neuromuscular function.[1] In patients with long term hospitalization various causes, like diabetes, inflammation, cancer, etc., can reduce the amount of vitamin B_1 and thiamine deficiency may develop. Thiamine deficiency results in impaired metabolism and oxidative stress that decreases survival rate of patients.[2] Therefore it is advisable to monitor the level of thiamine and its derivatives.

Whole scale of direct and indirect methods for the analysis of thiamine and its phosphorylated forms were developed as well as reviewed in the past.[3] Some methods are used in clinical practice, but do not meet the requirements needed for simple, fast, precise and accurate determination. Accordingly, the need of novel method that could overcome these problems is necessary.

The new method for the determination of thiamine and its derivatives suitable for clinical applications was developed. The main goal of this project was to optimize extraction procedure that would allow quantitative analysis of thiamine and its phosphorylated forms in human plasma. Several different extraction techniques have been tested with the effort to remove interfering compounds and to increase the sensitivity of the analysis. All reagents have been tested on native and spiked samples to obtain the best results. Up to now the most suitable technique with sufficient purity of sample seems to be the protein precipitation followed by centrifugation, filtration and derivatization. Several precipitation reagents have been tested. So far the best results were obtained using methanol: zinc sulfate mixture. Ultra-filtration and SPE was also tested but so far without satisfactory results. To find the most appropriate extraction procedure several other techniques will be tested in the future. After the optimization and miniaturization of extraction procedure the method will be prepared for further validation.

The study was supported by project SVV 260 184, PRVOUK P37/12 and University Hospital in Hradec Králové, IČO: 00179906.

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DEVELOPMENT OF UHPLC-UV METHOD FOR THE DETERMINATION OF OMEPRAZOLE IN ORAL SUSPENSIONS

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

[1] Department of Analytical Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[2] 3rd Internal Gerontometabolic Clinic, 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). PPIs block the secretion of gastric acid both selectively and irreversibly by inhibiting H^+/K^+ATPase in parietal cells. Omeprazole plays role in the treatment of the acid-related disorders, such as gastroesophageal reflux disease (GERD), Barrett's esophagus, peptic ulcers disease and Zollinger-Ellison syndrome.

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, elderly as well as critically ill patients having problems to swallow solid dosage form. Thus, individually prepared oral suspensions of omeprazole facilitate the administration and the dosage exactly in this group of patients.[1,2]

The aim of this project was development and validation of modern HPLC method for the omeprazole stability monitoring in these suspensions. The separation of omeprazole standard solution, omeprazole related compound (as impurity) and methylparaben (as internal standard) was performed by Kinetex™ C18 column (50 × 2.1 mm, 1.7 µm) using mobile phase 25 mmol/L phosphate buffer (pH 7.6) and acetonitrile (74:26, v/v). All analytes were determinated by UV detection at 300 nm. Method was partially validated.

The newly developed, simple and rapid method will be applied to the analysis of concentration of omeprazole and his stability in six different suspension formulations prepared in the Hospital pharmacy in Motol University Hospital and will serve for Department of Pediatrics, 2nd Faculty of Medicine, Charles University and Motol University Hospital.

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DEVELOPMENT OF HPLC METHOD FOR DETERMINATION OF EIGHT SYNTHETIC FOOD DYES: COMPARISON OF MONOLITHIC AND FUSED-CORE COLUMN

LHOTSKÁ, I., STACHOVÁ, I., ŠATÍNSKÝ, D.

Department of Analytical Chemistry, Faculty of Pharmacy in Hradec Králové,
Charles University, Czech Republic
e-mail: lhotski@faf.cuni.cz

Using of synthetic dyes in food industry is quite controversial. There is a positive list of permitted food colourings settled by EU legislation. Although these colours should be harmless, there are numerous studies warning of excessive consumption of synthetic colours in food.[1,2] In addition, they can cause some allergies and rashes in sensitive people.

Two HPLC methods for determination of these dyes were developed and validated, their separation efficiency and validation parameters were compared. The monolithic column Chromolith Performance CN 100 × 4.6 mm with guard column 5 × 4.6 mm (Merck) and fused-core particle column Ascentis Expres ES-CN, 100 × 4.6 mm, 5 μm (Sigma Aldrich) were chosen for separation. Different mobile phases, methanol or acetonitrile, and different buffers and their concentrations were tested for separation on both columns. The dyes were detected at three wavelengths according to their absorption maximum (420, 482 and 625 nm).

Final conditions of dyes separation, advantages and disadvantages of both approaches are going to be presented. Fused-core column showed higher separation efficiency including the shortening of analysis time nearly twice. On the other hand monolithic column showed lower back pressure and blank chromatogram with less interferences in case of some beverage matrices.

Finally, yellow, blue and green synthetic dyes were determined in fruit drinks, and in green beers in Easter seasons 2014 and 2015 to investigate their abuse.

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THE TESTING OF NANOFIBERS AS POTENTIAL SORBENTS IN SOLID PHASE EXTRACTION ON-LINE COUPLED TO HPLC

PARMOVÁ, M., CHOCHOLOUŠOVÁ, HAVLÍKOVÁ, L., ŠATÍNSKÝ, D.

Department of Analytical Chemistry, Faculty of Pharmacy in Hradec Králové,
Charles University, Czech Republic
e-mail: parmovam@faf.cuni.cz

Nowadays, SPE (solid phase extraction) is the most popular sample pre-treatment technique. Sorbents for SPE are still innovated and one of these innovations is using of

nanofibers as an extraction phase. Nanofibers can be defined as fibers with diameters less than 1000 nm. They have a large surface area and a great sorption capacity thanks to the extremely small fiber size. Electrospinning is one of the most conventional methods to produce nanofibers. Nanofibers are formed in electrostatic field from solution or melt of polymer. Electrospinning technology can be divided into two groups: needle electrospinning (known also as conventional electrospinning) and needleless electrospinning.[1] Polystyrene, polyamide 6, polyamide 66, and polypyrrole are the most often used polymers in the SPE nanofiber extraction.

Recent trends in sample pre-treatment are also focused on increasing the speed of analysis, the possibility of automation of processes and on-line connection of the preparation step with suitable analytical technique. Sample pre-treatment is realized in the HPLC system, in on-line connection and after extraction, analyte is directly injected to the analytical column.

This work is focused on the application of nanofiber polymers as sorbents in on-line SPE-HPLC. Tested analytes were chosen from the groups of pyrethroids and carbamates. These two groups of analytes have different physico-chemical properties and therefore different behavior in on-line SPE-HPLC system was observed. The conditions for SPE-HPLC (valve switching time, sample washing step, HPLC mobile phase composition) were optimized.

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PARALLEL ARTIFICIAL LIQUID MEMBRANE EXTRACTION – A NEW APPROACH FOR SELECTIVE SAMPLE PREPARATION

PILAŘOVÁ, V.,[1] SKOGLUND ASK, K.,[2] GJELSTAD, A.,[2] NOVÁKOVÁ, L.,[1] PEDERSEN-BJERGAARD, S.[2]

[1] Department of Analytical Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[2] School of Pharmacy, Faculty of Mathematics and Natural Sciences, University of Oslo, Norway
e-mail: pilarovv@faf.cuni.cz

Sample preparation is the key and the most time-consuming step of analytical method. It is crutial for removing interfering matrix components which can affect the results of analysis. In recent years, many different microextraction approaches based on solid phase extraction (SPE) and liquid liquid extraction (LLE) were developed as alternative strategies for sample pretreatment. The advantages of these microextraction techniques consist not only in use of small volumes of organic solvents and sample but also in shortening of sample preparation time. They can accelerate sample processing about tens of minutes. Parallel artificial membrane extraction (PALME) is one of these modern microextraction approaches. PALME can be viewed as a miniaturized version of LLE, where the analytes

in biological matrix pass through a porous membrane wetted by an organic solvent into a water acceptor solution. The goal of this study was to develop a liquid chromatography method using ion trap mass spectrometer for analysis and PALME for the isolation of polar basic drugs from human plasma. The separation was performed on HSS T3 (2.1 × 100 mm, 1.8 μm), using gradient elution with methanol and 20 mM formic acid with run time 11 minutes. Sample extraction was consisted of plasma dilution with phosphate buffer pH 7.0 to total volume 250 μL in the ratio 1:2 and PALME extraction. Polypropylene membrane with 2.5 μL 2-nonanone + 15% of diethylhexyl phthalate (DEHP) was used as an artificial membrane, 50 μL 150 mM trifluoroacetic acid was used as an acceptor solution. The extraction took 45 minutes. The method was validated in terms of precision, accuracy, range, linearity, limit of detection, limit of quantification and matrix effects at 4 concentration levels.

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NEW EXTRACTION SPECTROPHOTOMETRIC METHODS FOR THE DETERMINATION OF NITROGEN MUSTARD (HN-3) IN ACIDIC ENVIRONMENT

ROZSYPAL, T., HALÁMEK, E., KOBLIHA, Z.

NBC Defence Institute, University of Defence in Brno, Czech Republic
e-mail: tomas.rozsypal@unob.cz

Nitrogen mustards are compounds that have been used as active ingredients of pharmaceutical preparations. However their effects on human body take also negative consequences such as secondary leukemia genesis because of side DNA alkylation.[1] Tris(2-chloroethyl)amine (HN-3) is military interesting blistering nitrogen mustard that was produced in significant quantities during World War 2. In alkaline environment the alkylating properties are predominant but in acid its tertiary amine structure can be useful for detection and determination of the analyte. The aim of this study is to create and evaluate methods for extraction spectrophotometric determination of HN-3 in acidic environment in field conditions.

Nine dyes form the group of sulfophthaleins, antraquinone dyes and sulfonated azo dyes were suitable for ion pair formation with the analyte. Complexes were extracted from aqueous phase into non-polar solvent (chloroform) and measured spectrophotometrically against blank solutions. Optimization of the determination process covered measuring of absorption curves, optimal pH, ion-pair stoichiometry, optimal dye concentration excess, extraction time, calibration curves, extraction recoveries and distribution ratios.

Because of high polarity the HN-3 is rarely disposed for ion pair formation as tertiary amine. Best molar absorptivities were reached with Bromothymol Blue and Bromocresol Green (301 and 148 $l\,mol^{-1}\,cm^{-1}$ respectively). Highest extraction recoveries were recorded by Bromothymol Blue (0.97), Acid Blue 129 (0.64) and Bromocresol Green (0.61). Best

limits of detection of proposed methods were reached with Acid Blue 25 (36.4 μg ml^{-1}) and Bromothymol Blue (45 μg ml^{-1}).

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USING SOLID-PHASE MICROEXTRACTION FOR PLASMA PROTEIN BINDING STUDY OF DPC

REIMEROVÁ, P., STARIAT, J., KOVAŘÍKOVÁ, P.

Department of Pharmaceutical Chemistry and Pharmaceutical Analysis,
Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
e-mail: reimerop@faf.cuni.cz

Solid-phase microextraction (SPME) is a relatively novel technique suitable for extraction of analytes from complex matrices using a small amount of phase attached to a fiber. This method is nowadays thoroughly studied for its use in protein binding (PPB) studies of drugs for its low recovery and reduced matrix interference thanks to higher selectivity of the fiber coating.[1]

Di-2-pyridylketone-4-cyclohexyl-4-methyl-3-thiosemicarbazone (DpC) is novel and highly potent anticancer drug[2] currently under advanced preclinical development. Measurement of PPB is an indispensable part of discovery as well as clinical development of this novel drug. Nonspecific binding of DpC to various materials and its chelation ability complicates the use of standard approaches such as equilibrium dialysis and ultrafiltration as well as employment of metal-based commercially available SPME fibers. Hence the aim of this project was to prepare novel SPME fibers from material resistant to chelation and utilize them for the determination of plasma protein binding of DpC *in vitro*.

In this study silicone fibers were coated with Discovery® DSC-18 sorbent using polydimethylsiloxane (PDMS) as a glue. Development of the SPME method included mainly optimization of extraction and desorption conditions. Potential carry over, matrix effect (PBS and plasma), reproducibility, linearity, accuracy and precision of the method were determined. Optimized procedure was then utilized for determination of PPB[3] of DpC at different concentrations in rat plasma *in vitro*. All samples were analyzed using UHLC-MS/MS to reach sufficient sensitivity for analysis of DpC *in vitro*.

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DEVELOPMENT OF HPLC-DAD METHOD FOR ANTHOCYANIN DETERMINATION IN DIFFERENT HIGHBUSH BLUEBERRY CULTIVARS

ŠMÍDOVÁ, B.,[1,2] DOSTÁLOVÁ, K.,[1] ŽIDOVÁ, P.,[2] ŠATÍNSKÝ, D.,[1] MATĚJÍČEK, A.,[2] PAPRŠTEIN, F.[2]

[1] Department of Analytical Chemistry, Faculty of Pharmacy in Hradec Králové,
Charles University, Czech Republic
[2] Department of Gene Pool, Research and Breeding Institute of Pomology Holovousy Ltd., Czech Republic
e-mail: smidovb1@faf.cuni.cz

Anthocyanins belong to the most important pigments of the vascular plants which are responsible for many colors in some flowers and fruits. Significant property of anthocyanins is their antioxidant activity which plays important role in prevention of many diseases.[1] High amounts of anthocyanins were found in highbush blueberries (*Vaccinium corymbosum* L.).

The aim of this research was to develop and optimize HPLC method for relatively fast separation and quantification of anthocyanins in highbush blueberry samples. 22 cultivars of highbush blueberry were tested. Anthocyanins were extracted into methanol with the addition of formic acid and then separated using Shimadzu HPLC system equipped with a diode array detector. Compounds were detected at wavelength of 520 nm. Different columns were tested, however column Kinetex PFP (150 × 4.6 mm, 2.6 μm) with guard column Ascentis® Express F5 (5 × 4.6 mm, 5 μm) was finally chosen. Columns were operated at 50 °C. Water solution of formic acid (solution A) and acetonitrile (solution B) were used as the mobile phases. Gradient elution mode was applied. Final time of separation was under 20 minutes. Correlation coefficient in concentration range between 1–100 mg L^{-1} was higher than 0.9991. Repeatability in three concentration levels (5, 20, 100 mg L^{-1}) ranged between RSD 0.21–0.98%. Significant differences in the representation of individual anthocyanins were demonstrated in the observed cultivars.

The study was supported by the NP MZe 20139/2006-13020 and CZ.1.05/2.1.00/03.0116.

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MOLECULARLY IMPRINTED POLYMERS FOR SELECTIVE EXTRACTION OF LOVASTATIN FROM FOOD SAMPLES AND ELIMINATION OF MATRIX EFFECTS

SVOBODA, P., SANDER, D., NOVÁKOVÁ, L.

Department of Analytical Chemistry, Faculty of Pharmacy in Hradec Králové,
Charles University, Czech Republic
e-mail: svobodp1@faf.cuni.cz

Lovastatin belongs to the group of cholesterol-lowering drugs and represents the first marketed statin. It is also the only statin that occurs in nature and found application in

clinical practice. This compound is produced by several fungal species of the genus *Trichoderma*, *Monascus*, *Penicillium* and *Aspergillus*. Lovastatin occurs in traditional Chinese food such as Pu-erh tea, Red yeast rice and Oyster mushroom. Liquid chromatography coupled to mass spectrometry (LC-MS) was used for its determination in the food samples.

LC-MS method is well-established and widely used analytical tool in the field of quantitative and qualitative analysis. It is characterized by high selectivity, sensitivity and robustness. However, this method suffers from a major drawback represented by matrix effects. They are caused by co-eluting compounds that affect the process of ionization in the ion source of mass spectrometer. They lead to signal suppression or enhancement and affect important parameters of LC-MS method. Food samples such as tea leaves, mushrooms and fermented rice are complex matrices prone to this undesirable phenomenon.

SPE sorbent based on the technique of molecularly imprinted polymers (MIP) was employed for sample preparation step due to its ability to selectively retain the target analyte and purify the complex samples. The tailor-made cavities for selective recognition of lovastatin were prepared using simvastatin as a template molecule, methacrylic acid as a functional monomer and ethylene glycol dimethacrylate as a cross-linker. Process of polymerization was initiated by azobisisobutyronitrile. Non-imprinted polymer (NIP) was prepared according to the same procedure as MIP, except that no template was used for its synthesis. NIP was employed as a control material for selectivity evaluation due to the absence of cavities. The resulting MIP material was completely characterized in terms of capacity, selectivity, repeatability of extraction procedure and reproducibility of synthesis. The optimized SPE procedure employing MIP sorbent successfully removed matrix effects in all food matrices.

STABILITY OF CARFILZOMIB – UHPLC-PDA-QT OF STUDY OF A PROTEASOME INHIBITOR

ŠESTÁK, V.,[1] ROH, J.,[2] KLEPALOVÁ, L.,[1] KOVAŘÍKOVÁ, P.[1]

[1] Department of Pharmaceutical Chemistry and Pharmaceutical Analysis,
Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[2] Department of Inorganic and Organic Chemistry, Faculty of Pharmacy in Hradec Králové,
Charles University, Czech Republic
e-mail: sestv7aa@faf.cuni.cz

Carfilzomib is an irreversible inhibitor of the protasome – a breakthrough target in cancer treatment.[1] Carfilzomib contains several structural features that might raise stability concerns, such as: 1) peptide bonds, 2) an epoxide, 3) a morfolin ring, 4) four stereogenic centres. Since this drug was granted an accelerated approval by FDA in 2012, only scarce data on analysis and none on stability of carfilzomib are available in the literature. Therefore our goal was to develop and validate a stability-indicating method to provide insight into the inherent stability of this new drug.

We developed and validated (according to ICH guidelines[2]) a novel method for quantitation of carfilzomib using a Nexera® UHPLC system with a PDA detector (Shimadzu, Japan), a C18-bearing silica type C column (Cogent Bidentate C18; 2.1 × 100 mm; 2.2 μm; 120 Å)

and acetonitrile/ammonium formate mixture in gradient mode. This method was used in investigation of carfilzomib's degradation kinetics. Additionally, we employed a Synapt® G2Si QTOF instrument (Waters, United Kingdom) in MS/MS elucidation of the chemical structures of degradation products, that were formed in a forced degradation study.

We found that carfilzomib is: 1) stable at neutral and slightly acidic pH, while it degrades in both low and high pH, 2) acceptably stable in pharmaceutical formulation but is 3) prone to oxidation and photodegration. The decomposition products resulted from peptide bond hydrolysis, epoxide hydrolysis, hydrogen chloride addition, base-catalyzed Robinson-Gabriel reaction, tertiary amine oxidation and isomerization.

Our results document the stability of carfilzomib and provide first information about identity of its degradation products. These results highlight the stability issues that need to be kept in mind for handling/storing.

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A NOVEL APPROACH TO THE LAB-IN-SYRINGE TECHNIQUE. DETERMINATION OF AMMONIA IN RIVER WATERS

ŠRÁMKOVÁ, I.,[1] HORSTKOTTE, B.,[1] SKLENÁŘOVÁ, H.,[1] SOLICH, P.,[1] KOLEV, S.[2]

[1] Department of Analytical Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[2] School of Chemistry, The University of Melbourne, 3010 Victoria, Australia
e-mail: sramkovai@faf.cuni.cz

A novel approach to the Lab-In-Syringe technique, also known as In-Syringe Analysis[1] is presented. It is based on using a secondary entrance to the syringe void through the modified syringe piston. This innovation allows straightforward automation of single-drop microextraction by simplifying the control of drop handling as well as in-drop analyte quantification.

The syringe pump was used in upside-down orientation. Sample homogenization, head-space enrichment by analyte, and syringe cleaning was facilitated by a magnetic micro-stirring bar placed inside the syringe and driven by an external rotating magnetic field.[2]

The system was characterized by the development of a sensitive method for ammonium determination in river and harbour waters. The method is based on head-space extraction of ammonia into a single drop of bromothymol blue indicator formed inside the syringe and on-drop sensing of the colour change using fibre optics. A repeatability of < 5% RSD, a linear range up to 25 μmol L^{-1}, and a limit of detection of 1.5 μmol L^{-1} were achieved. Study of interferences proved excellent robustness of the method towards humic acid,

salts, and detergents, thus being superior to state-of-the-art gas-diffusion approaches. A mean analyte recovery of 106.14% was found analysing spiked water samples.

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SEQUENTIAL INJECTION MANIFOLD AS A TOOL FOR AUTOMATED PERFORMANCE OF DRUG PERMEATION STUDIES

ZELENÁ, L.,[1] HYRŠOVÁ, L.,[2] FIBIGR, J.,[1] PÁVEK, P.,[2] SKLENÁŘOVÁ, H.[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: zelenal@faf.cuni.cz

Sequential injection analysis (SIA) is a second generation of analytical flow techniques introduced by prof. Růžička in 1990.[1] Its universal set-up allows to build an easy manifold to perform sample treatment such as dilution, derivatization, extraction or separation and its detection in one system. SIA manifold is also used for long-term monitoring applications, such as dissolution tests of tablets, liberation tests of ointments or permeation tests of drugs.

Drug permeation studies[2] are tests used for evaluation of drug transport through cellular monolayer, drug interaction with membrane transporters or drug-drug interactions.

This experimental work describes analytical system composed of SIA manifold connected with a Franz diffusion cell as a liberation unit used for drug transport monitoring. P-glycoprotein (P-gp, MDR1) transporter function was evaluated using Rhodamine 123 as a marker of transport and verapamil hydrochloride as a P-gp inhibitor.

Description of flow-based apparatus, its comparison with batch-wise apparatus and experimental data obtained during permeation tests with Rhodamine 123 and verapamil on cell line MDCKII-MDR1 will be presented.

The study was supported by the Grant Agency of the Charles University, project GAUK No. 159415, and by the project of specific research, SVV No. 260184.

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THE INFLUENCE OF MAGNESIUM STEARATE ON THE FLOW AND SHEAR PROPERTIES OF SORBITOL

HURYCHOVÁ, H.,[1] STONIŠ, J.,[1] SVĚRÁK, T.,[2] ŠKLUBALOVÁ, Z.[1]

[1] Department of Pharmaceutical Technology, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[2] Institute of Materials Science, Faculty of Chemisty, Brno, University of Technology, Czech Republic
e-mail: hurychh@faf.cuni.cz

The flow properties of powders are very important for handling, transport, storage and the correct dosage of active ingredients and excipients in production of solid dosage forms. The flow, shear and avalanching behaviour of powders depend on the internal friction between grains of the material.[1,2] For evaluation of flow properties, the standard pharmacopoeial methods are used out of them the measurement of the mass flow rate is the most frequent. To measure the angle of internal friction, the shear tester is useful.[3] To increase the flow of powders, glidant such as Magnesium stearate is usually used; but it also can be used as a lubricant which prevents adhesion of tablets on the die or punch wall.[4]

This work studies the influence of the concentration of magnesium stearate MgSt (0.5 wt% or 1.0 wt%, respectively) on the mass flow rate Q (g/s) of sorbitol for direct compression through a circular hopper orifice having a diameter of 1.0 cm. The shear properties of sorbitol and mixtures with MgSt were studied using Jenike shear tester.

The very low values of the cohesion for sorbitol were detected infering the non-cohesive, free-flowing material properties. MgSt increased the mass flow rate and decreased the cohesion properties. The better results were obtained with 0.5 wt% addition of MgSt.

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EVALUATION OF TRANSDERMAL PERMEATION ENHANCERS BASED ON MONOSACHARIDES

KOPEČNÁ, M.,[1] MACHÁČEK, M.,[2] ROH, J.,[1] VÁVROVÁ, K.[1]

[1] Department of Inorganic and Organic Chemistry, 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: kopem7ba@faf.cuni.cz

Transdermal route of drug administration is a non-invasive technique with many advantages, compared to other drug delivery routes. However, skin acts as formidable barrier preventing majority of environmental substances from entering into organism. One of the methods how to overcome this barrier is to use permeation enhancers, substances which by various mechanisms reversibly decrease barrier properties of skin. In this study we studied effect of monosaccharide derivatives and their analogues on permeation of two model drugs theophylline (TH) and cidofovir (CDV) through human skin *in vitro*. Afterwards, toxicity, reversibility and mode of action of selected enhancers were studied. The *in vitro* permeation experiments on human skin with model drug theophylline (TH) revealed, that the best activity possess substances 91.1 and 94.1 which significantly enhanced flux of TH 5.9 and 8.3 times, respectively, compared to control. Although none of 91.1 and 94.1 enhanced flux of CDV through human skin, both of them significantly increased CDV concentration in epidermis, with values 7.4 and 6.9 times higher, respectively, compared to control. To determine possible mechanism of action of these enhancers were used infrared studies on isolated human stratum corneum. Results suggested that the mechanism of action of both 91.1 and 94.1 probably involves interaction with barrier lipids. Reversibility of action of selected substances (91.1 and 94.1) after 24h application on human skin was proved using transepidermal water loss measurements. Finally, we studied toxicity of 91.1 and 94.1 on two cell lines; Swiss albino mouse embryonic fibroblasts (3T3) and spontaneously immortalized human keratinocytes (HaCaT). IC_{50} values for substance 91.1 on 3T3 and HaCaT were 25.18 ± 2.79μM and 24.35 ± 0.96μM, respectively. Due to low solubility of 94.1 we were only able to determine that its IC_{50} concentration value is higher than 60μM on both 3T3 and HaCaT cell lines.

The study was supported by Charles University (GAUK 1404213 and SVV 260 183) and the Czech Science Foundation (13-23891S).

BIORELEVANT *IN-VITRO* RELEASE TESTING METHODS FOR CONTROLLED RELEASE PARENTERALS: AN INTRODUCTION

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

[1] Department of Pharmaceutical Technology, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[2] Department of Pharmaceutical Technology and Biopharmaceutics, Institute of Pharmacy, University of Bonn, Bonn, Germany
e-mail: kozakja1@faf.cuni.cz

Controlled release parenteral preparations (microparticles, implants) provide several advantages in comparison with other dosage forms. Despite the rapid development in this area, there is no compendial *in-vitro* release testing apparatus specifically designed for these types of devices. *In-vitro* release tests are irreplaceable tools for characterisation of dosage forms. They provide valuable information about the formulation variables and can predict performance of the device *in-vivo*. The purpose of "biorelevant" *in-vitro* methods is to include (and to recognize) factors which have influence on drug release *in-vivo*. Development in the field of biorelevant dissolution has been predominantly focused on oral dosage forms. To simulate environment at the site of administration in case of implantable devices, use of hydrogels have been suggested. In our proposed novel setup, the controlled release device is incorporated in thin agarose hydrogel and placed in phosphate buffer (pH 7.4). Therefore the possibility of sampling from buffer and use of conventional analysis methods is retained. Initial results are also presented, although more experiments will be needed to make any conclusions.

The study was supported by SVV 260 183.

OPTIMIZATION OF FABRICATION OF NANOPARTICLES WITH HYDROPHILIC BIOLOGICALLY ACTIVE SUBSTANCES

NGUYENOVÁ, J., DITTRICH, M., ŠNEJDROVÁ, E.

Department of Pharmaceutical Technology, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
email: nguyenj@faf.cuni.cz

Nanoparticles have been developed as an important strategy to deliver conventional drugs, recombinant proteins, vaccines and more recently, nucleotides.[1] Nanoencapsulation of drugs through various methods increases drug efficacy, specificity, tolerability and therapeutic index of corresponding drugs.[2]

Although the definition identifies nanoparticles as having dimensions below 0.1 μm or 100 nm, especially in the area of drug delivery relatively large (size above 100 nm) nanoparticles may be used for loading a sufficient amount of drug onto the particles.[3]

The objective of this project is to study and optimize methods of preparation of medicated polymeric nanoparticles from aliphatic hydroxyacids as carriers. Nanoparticles will

contain hydrophilic lowmolecular weight, oligomeric, and polymeric active substances. The aim is to minimize mean size and size polydispersity and to maximize yield and encapsulation efficiency. Efforts will be directed to development of robust methods of preparation of standardised products.

Select samples will be tested in cooperation with The Department of Pharmacology and Toxicology for relevant interactions with biological systems.

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A BIOPHYSICAL INSIGHT INTO A DISTURBED SKIN LIPID BARRIER IN B-GLUCOCEREBROSIDASE DEFICIENCY

SOCHOROVÁ, M., STAŇKOVÁ, K., PULLMANNOVÁ, P., KOVÁČIK, A., VÁVROVÁ, K.

Department of Inorganic and Organic Chemistry, Faculty of Pharmacy in Hradec Králové,
Charles University, Czech Republic
email: sochormi@faf.cuni.cz

Glucosylceramides (GCer) are precursors for all ceramide (Cer) types in the stratum corneum (SC) and their hydrolysis by β-glucocerebrosidase (GCerase) is important in the formation of the epidermal permeability barrier.[1] Decrease in Cer content in patients with Gaucher disease is related to decreased GCerase activity.[2] We studied how the presence of GCer and/or lack of Cer influences permeability barrier properties of model SC lipid membranes.

The SC model membranes were prepared as an equimolar mixture of Cer or GCer in different ratios, Chol, FFA and 5% of CholS. Also membranes with decreased Cer fraction were prepared. Four permeability markers – water loss through the membrane (TEWL), opposition to electrical current and steady state fluxes of theophylline and indomethacin, were evaluated.

The replacement of 5–25% of hCer by GCer led to impairment of the permeability of the prepared membranes to all 4 permeability markers. At these concentrations, the presence of GCer is a stronger contributor to this disturbance than a lack of hCer. The reduction of hCer to 50 or 0% showed that the lack of hCer disturbs the barrier, while the larger GCer/hCer ratio or complete replacement of hCer by GCer has no negative effects on permeability.

In conlusion, we confirmed that the accumulation of free GCer associated with their incomplete processing contributes to altered permeability barrier properties in skin disorders. However, this barrier perturbation by free GCer seems to be concentration-dependent.

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INFLUENCE OF SUPERDISINTEGRANTS ON THE PROPERTIES OF TABLETS COMPOSED FROM DIFFERENT STARCHES

STONIŠ, J., HURYCHOVÁ, H., SKOŘEPOVÁ, Z., ONDREJČEK, P., SVAČINOVÁ, P., ŠKLUBALOVÁ, Z.

Department of Pharmaceutical Technology, Faculty of Pharmacy in Hradec Králové,
Charles University, Czech Republic
e-mail: stonisj@faf.cuni.cz

Orodispersible tablets are uncoated tablets intended to be placed in the mouth, where they disperse rapidly.[1] Quick disintegration and sufficient radial strenght are two out of the most important properties of them.[2] In this study, the properties of four tablet mixtures prepared from granules made by fluid bed granulation technique were studied.

To prepare the granules, potato starch (PS) and corn starch (CS) as fillers, respectively, and sodium starch glycolate (SSG) or cross-carmellose (CCMC) as superdisintegrants, respectively, were used; povidone (PVP) 10% served as a binder. Granules were evaluated for their particle size distribution, flow properties, compressibility properties and particle density. Then, magnesium stearate (0.5%) was added as a glidant and tablets of 7 mm in diameter and approximately 0.1 g in mass were compressed using the different compression forces to achieve the starting radial strenght of 1 MPa. The diameter and the height of tablets was measured as well as the crushing strenght and the disintegration time.

Results of this study proved, that out of the tested combinations, PS CCMC granules served the best flow and tablet properties. The granules had satisfactory particle size distribution and the mean particle size x_{50} (180 μm), the appropriate angle of repose (39°) as well as the compresibility index (18.14%). Tablets made from PS CCMC had sufficient radial strenght (0.61 MPa) and disintegrated within two minutes.

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DIFFERENT SUBSTITUTIONS OF ISOFLAVONOIDS CORE INFLUENCE MARKEDLY THEIR ANTIPLATELET POTENTIAL

APPLOVÁ, L.,[1] ŘÍHA, M.,[1] KARLÍČKOVÁ, J.,[2] FILIPSKÝ, T.,[1] MACÁKOVÁ, K.,[2] HRDINA, R.,[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 and Ecology, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
e-mail: applovl@faf.cuni.cz

Beneficial effects of isoflavonoids on the human health, in particular the lower risk of the coronary artery disease was reported.[1] The aim of this study was to compare antiplatelet activities of 17 isoflavonoids and to find the mechanisms of their action.

The screening in the whole human blood has shown that 12 isoflavonoids blocked platelet aggregation induced by arachidonic acid. The structure-activity relationship revealed that 7,4′-dihydroxyl group represented the most efficient functional group. Substitution of these positions by a methoxyl group led to a reduction of the effect while glucose in position C-7 was associated with almost complete loss of the activity. Presence of a 5-hydroxyl group seemed to be beneficial, as well as, the co-presence of a 6-methoxy group. The latter was associated with higher effect than the standard antiplatelet drug, acetylsalicylic acid (ASA). Active isoflavonoids acted as antagonists on thromboxane A_2 receptors and inhibitors of cyclooxygenase-1.

Isoflavonoids with appropriate chemical structure possess even higher anti-platelet activity in comparison with ASA[2] and their advantage over this standard drug might be associated with simultaneous influence on two steps on the platelet aggregation.

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^{61,64}Cu-(SCN)-PHOSPHINATE-IMMUNOGLOBULIN-G M75 AND THEIR BIOLOGICAL TESTING

ČEPA, A.,[1,2] RÁLIŠ, J.,[2] MAREŠOVÁ, L., KLEINOVÁ, M., SEIFERT, D.,[2] SIEGLOVÁ, I.,[4] KRÁL, V.,[4] LEBEDA, O.,[2] LÁZNÍČEK, M.[1]

[1] Department of Pharmacology and Toxicology, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[2] Department of Radiopharmaceuticals, Nuclear Physics Institute of the CAS, v. v. i., Czech Republic
[3] ÚJV Řež a. s., Czech Republic
[4] Laboratory of Structural Biology, Institute of Molecular Genetics of the CAS, v. v. i., Czech Republic
e-mail: cepaa7aa@faf.cuni.cz

The work was focused on a new approach to the labelling of antibody IgG M75 for epitope human carbonic anhydrase IX with copper radioisotopes. Human carbonic anhydrase IX is a membrane enzyme that is significantly expressed in some types of hypoxic cancer cells and copper radioisotopes offer wide range of diagnostic, therapeutic and theragnostic properties.[1] The antibody IgG M75 was successfully conjugated with "phosphinate", recently developed, non-commercial copper-specific chelator. The conjugation method was optimized and provides well-reproducible results. The conjugate was then labelled with two copper radioisotopes: ^{61}Cu (3.339 h) and ^{64}Cu (12.701 h). The resulting labelled conjugates were tested *in vivo* in mice with inoculated colorectal cancer.[2] Obtained data suggest that the prepared labeled antibody is a good candidate for diagnostics of some hypoxic solid tumors by imaging via positron emission tomography (PET).

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THE INCIDENCE OF DYSRHYTHMIAS AFTER ADMINISTRATION OF ANTIPSYCHOTIC OLANZAPINE

GULAČ, P., VICEN, M., STANKOVIČOVÁ, T.

Department of Pharmacology, Faculty of Pharmacy, Comenius University Bratislava, Slovac Republic
e-mail: patrik.gulac@gmail.com

Psychosis is a mental disease with increased risk of developing cardiovascular diseases as well as increased risk of mortality after administration of antipsychotics. Aim of the work was to analyze the effect of antipsychotic olanzapine (OLA 10 mg/kg s.c.) on isolated spontaneously beating rat heart. We used perfusion according to the Langendorff to investigate the effect of OLA. We perfused rat heart for 20 minutes by Krebs-Henseleit solution

followed by 30 min stop-flow ischemia and 45 min. reperfusion (IR injury). Analysis of ECG during reperfusion showed longer QT and QTc intervals durations in the group pre-medicated with OLA. As well as increased incidence of reperfusion-induced dysrhythmias in order ventricular premature beats > bigeminies > trigeminies > salvos. Administration of OLA caused spontaneously terminating episodes of ventricular tachycardia in a time between 10th and 25th minute in reperfusion. Average incidence of ventricular tachycardia during whole reperfusion was 1.6 episodes and duration was 31.5 second per one heart. This represents an increase compared to the IR injury group by 60 percent in the number of episodes and the doubling of their average duration. OLA modulated the activity of the isolated spontaneously beating rat heart and displayed proarrhythmogenic effect during reperfusion.

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RADIOLABELING OF ANTI-VEGFR2 MONOCLONAL ANTIBODY RAMUCIRUMAB WITH TECHNETIUM-99M

JANOUŠEK, J.,[1] BÁRTA, P.,[2] TREJTNAR, 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
e-mail: janousj2@faf.cuni.cz

Vascular endothelial growth factor (VEGF) is one of the most important regulators of angiogenesis, including tumour angiogenesis. VEGF binds on two types of receptors with tyrosine-kinase activity of which VEGF receptor 2 (VEGFR2) is a key receptor in tumour neoangiogenesis.[1] Ramucirumab (RAM), is a novel therapeutic monoclonal anti-VEGFR2 antibody with much higher affinity than its natural ligand.[2] The aim of this work was to evaluate a possibility of labeling RAM with technetium-99m and verify radiochemical purity and stability of radiolabeled antibody. For labeling of RAM with ^{99m}Tc, we optimized a direct method based on reduction of disulfide bridges in antibody molecule with 2-mercaptoethanol[3] using many modifications of the experimental conditions. The radiochemical purity of labeled antibody was assayed by instant TLC on silica gel (ITLC-SG). The sample was analyzed at various times by SE-HPLC with radiometric detection to evaluate a stability of labeled antibody. The introduced methods enable effective labeling of RAM with ^{99m}Tc and result in sufficiently stable radiopreparation. The developed radiolabeled preparation may be used in the follow-up studies to evaluate biological behavior of ^{99m}Tc-RAM *in vitro* and *in vivo*.

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SOLUBLE ENDOGLIN EFFECTS IN AORTA FROM MICE FED HIGH FAT DIET

JEŽKOVÁ, K.,[1] RATHOUSKÁ, J.,[1] NĚMEČKOVÁ, I.,[1] FIKROVÁ, P.,[1], DOLEŽELOVÁ, E.,[1] VAŘEJČKOVÁ, M.,[1] VITVERIVA, B.,[1] TYSONOVÁ, K.,[1] SERWADCZAK, A.,[2] BERNABEU, C.,[3] LOPEZ-NOVOA, J. M.,[4] CHLOPICKI, S.,[2] NACHTIGAL, P.[1]

[1] Department of Biological and Medical Sciences, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[2] Jagiellonian Centre for Experimental Therapeutics (JCET), Krakow, Poland
[3] Center for Biological Research, Spanish National Research Council (CSIC), and Biomedical Research Networking Center on Rare Diseases (CIBERER), Madrid, Spain
[4] Renal and Cardiovascular Physiopathology Unit, Department of Physiology and Pharmacology, University of Salamanca, Spain
e-mail: jezkk6aa@faf.cuni.cz

A soluble form of endoglin (sEng) is generated by the cleavage of its extracellular domain during development of some pathological conditions. Several authors have suggested the participation of sEng in the mechanisms of endothelial dysfunction. Thus, we tested the hypothesis that high plasma concentration of sEng in *Sol-Eng*$^+$ mice combined with high fat diet might contribute to the development of endothelial dysfunction.

Six-month-old female transgenic mice overexpressing human sEng with low (*Sol-Eng*$^+$ *low*) or high levels of soluble endoglin (*Sol-Eng*$^+$ *high*) were fed a high fat rodent diet containing 1.25% of cholesterol and 40% of fat for 3 months. The expressions of pro-inflammatory P-selectin, ICAM-1, pNFkB, COX-2, and oxidative stress-related markers HO-1, NOX-1 and NOX-2 in aortas of *Sol-Eng*$^+$ *high* mice were significantly higher than in *Sol-Eng*$^+$ *low* mice. Surprisingly endothelium-dependent response induced by acetylcholine was preserved in *Sol-Eng*$^+$ *high* mice compared to *Sol-Eng*$^+$ *low* mice.

These results suggest that high concentrations of sEng in plasma induce the activation of pro-inflammatory, pro-oxidative as well as vasoprotective mechanisms in the vessel wall.

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DIFFERENTIATION AND EPIGENOME AFFECTING DRUGS CHANGE EXPRESSION OF NUCLEOSIDE TRANSPORTERS IN PLACENTAL Bewo CELL LINE

JIRÁSKOVÁ, L., ČERVENÝ, L., ŠTAUD, F.

Department of Pharmacology and Toxicology, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
e-mail: jiraskl2@faf.cuni.cz

Nucleoside transporters (NTs) participate in nucleoside uptake by the placenta and contribute to transplacental permeation of nucleoside derived drugs. Evidence on regulation of placental NTs is sparse, therefore this project was aimed to analyze effect of epigenome (butyrate sodium, valproic acid sodium salt and 5-azacytidine) and differentiation (forskolin) affecting drugs on mRNA expression of genes encoding two subfamilies of NTs, i.e. equilibrative nucleoside transporters (ENT1, ENT2) and concentrative nucleoside transporters (CNT2, CNT3) in human choriocarcinoma derived BeWo cell line.[1,2]

Using qRT-PCR we found that only forskolin, cAMP-dependent protein kinase A (PKA) activator that induces syncytiotrophoblast formation in BeWo cells, had significant effect on the expression of *CNT2*. Upregulation of CNT2 in presence of forskolin was reversed by simultaneous application of KT5720, a model PKA inhibitor. Furthermore, significant increase of adenosine, a model substrate of CNT2, uptake was observed, showing higher mRNA expression *CNT2*, is reflected also in its elevated function. Forskolin-induced placentation of BeWo cell line was confirmed by mRNA analysis of endogenous retroviral envelope gene syncytin-1 (HERVW1), product specifically expressed in placental trophoblasts. Interestingly, mRNA expression analysis of *CNT2* mRNA in the first- and third-trimester human placenta also revealed significantly increasing tendency.[3]

In conclusion we propose that placental NTs are not epigenetically regulated. On the other hand expression/function of CNT2 seems to be PKA dependent and changes during placentation. We presume this phenomenon might also occur in the human placenta. As placenta is highly dependent on exogenous supply of pyrimidine nucleosides, we suggest that upregulation of CNT2 during placentation/gestation may be thus attributed to higher placental demands of nucleosides during gestation. It can also be predicted that transplacental permeation of CNT2 substrates increases during gestation.

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RIBAVIRIN TRANSPORT ACROSS PLACENTA AND THE ROLE OF NUCLEOSIDE TRANSPORTERS

KARBANOVÁ, S., ČERVENÝ, L., PTÁČKOVÁ, Z., ŠTAUD, F.

Department of Pharmacology and Toxicology Faculty of Pharmacy in Hradec Králové,
Charles University, Czech Republic
e-mail:karbanos@faf.cuni.cz

Ribavirin is highly used hydrophilic nucleosid-derived antiviral drug. It is currently recommended for combination therapy of hepatitis C virus (HCV) or HCV/HIV infection. *In vitro* and *in vivo* evidence on teratogenicity or embryocidal effect of ribavirin have shown potential risks, thus considering gestation as contraindication of ribavirin prescription. Ribavirin is suggested substrate of nucleoside transporters (NTs). NTs are divided in two subfamilies – equillibrative nucleoside transporters (ENTs) and concentrative Na^+ dependent nucleoside transporters (CNTs). In the placenta S-(4-Nitrobenzyl)-6-thioinosinu (NBMPR) sensitive ENT1 and NBMPR insensitive ENT2, and CNT2 are most abundantly expressed.[1]

In our study we aimed to determine whether NTs participate in transplacental passage of ribavirin. For this purpose we employed i) an *in situ* dually perfused rat term placenta model, analyzing materno-fetal (M-F) and feto-maternal (F-M) transplacental clearances of ribavirin on the organ level and ii) an *ex vivo* uptake experiment in fragments of human placental fresh villous tissue.

Ribavirin M-F and F-M clearances showed low level of its transplacental permeation, with negligible placental accumulation after the perfusion (≤3% of the ribavirin dose) NBMPR (0.1 μM and 100 μM) decreased ribavirin clearance in both directions to comparable level. Importantly, exposure to NBMPR (100 μM) and/or depletion of Na^+ in buffer resulted in inhibited ribavirin uptake by human fresh villous fragments.

In conclusion, our data document involvement of ENTs and CNTs in transplacental permeation of ribavirin. ENT1 and CNT2 are supposed to be predominant in placenta but further studies should be carried out to specify type NTs involved in ribavirin transplacental transport.

The study was supported by GAUK 324215/C/2015 and SVV/ 2015/260-185.

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INTERACTIONS OF INTESTINAL INFLUX TRANSPORTER hOATP1A2 WITH SELECTED FLAVONOIDS

NAVRÁTILOVÁ, L., RAMOS MANDÍKOVÁ, J., TREJTNAR, F.

Department of Pharmacology and Toxicology, Faculty of Pharmacy in Hradec Králové,
Charles University, Czech Republic
e-mail: navratl2@faf.cuni.cz

The bioavailability of orally administered compounds could be influenced by intestinal transporters. The major human intestinal transporters for uptake of organic anions are organic anion transporting polypeptides OATPs. In this study we focused on hOATP1A2 which is expressed in the apical membrane of human enterocytes. Some dietary flavonoids have been shown to inhibit human OATP subtypes involved in the drug intestinal absorption.[1] Therefore, the potential interactions of flavonoids with hOATPs should be considered as a factor potentially affecting pharmacokinetics of relevant drugs.

The aim of this study was to assess the potential inhibition of hOATP1A2 by natural compounds from the group of flavonoids (quercetin, myricetin, galangin, pinobanksin, pinocembrin, chrysin, fisetin) *in vitro*.

HEK293 cells transiently transfected with hOATP1A2 were used as the experimental model. The cells transfected with the empty vector served as a control. Inhibitory studies measuring hOATP1A2-mediated uptake of the typical substrate, radiolabelled [^{3}H]-estrone 3-sulfate, were employed to determine the inhibitory potency of flavonoids. Quercetin, the known OATP inhibitor, served as a comparator.

All mentioned flavonoids showed the statistic significant inhibition of the hOATP1A2-mediated uptake. The most potent hOATP1A2 inhibitors seem to be fisetin and pinocembrin with IC_{50} of 0.2 μM and 2.0 μM, respectively.

According to the obtained results, these natural compounds could potentially affect in varying degrees uptake of the drug substrates transported by human OATP1A2 (e.g. statins) into the enterocytes. So there is a possibility of food-drug interactions in humans at the level of absorption.

The study was supported by grant of the Czech Science Foundation (GAČR) no. 303/12/G163 and Charles University (SVV 260185 and PRVOUK P40).

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THE HAEMODYNAMIC EFFECTS OF FLAVONOID METABOLITE 3-(3-HYDROXYPHENYL)PROPIONIC ACID IN RAT

POUROVÁ, J., NAJMANOVÁ, I., VOPRŠÁLOVÁ, M., MLADĚNKA, P.

Department of Pharmacology and Toxicology, Faculty of Pharmacy in Hradec Králové,
Charles University, Czech Republic
e-mail: jana.pourova@faf.cuni.cz

The flavonoid intake is supposed to be associated with a lower cardiovascular mortality.[1] Paradoxically, the oral bioavailability of flavonoid aglycones is low.[2] The aim of this study was to test whether metabolites formed in the gastrointestinal tract by microflora could contribute to the effect.[3] A series of quercetin metabolites formed by both human enzymes and colon microflora were tested *in vitro* on isolated rat aortic rings precontracted with norepinephrine. A number of them caused vasodilatation with 3-(3-hydroxyphenyl) propionic acid (3HPPA) being clearly the most potent one. The vasodilatory activity of 3HPPA was confirmed by *in vivo* experiments on both normotensive and spontaneously hypertensive rats. Subsequent experiments indicated that arterial blood pressure decrease after 3HPPA was caused by the peripheral effect of the compound on vascular beds and could be NO-based. This is the first study showing that a metabolite of flavonoids formed by human microflora has haemodynamic effect.

The study was supported by the Charles University (grant No. 253115 C, the program "Research and Study of the Drugs" (PRVOUK P40)) and by the The Czech Science Foundation (project No. P303/12/G163).

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INTERACTIONS OF ANTIRETROVIRALS WITH THE MAIN PLACENTAL TRANSPORTERS

PTÁČKOVÁ, Z.,[1] ČERVENÝ, L.,[1] ČEČKOVÁ, M.,[1] ŘEZNÍČEK, J.,[1] KARBANOVÁ, S.[1]
GREENWOOD, S. L.,[2] ŠTAUD, F.[1]

[1] Department of Pharmacology and Toxicology, Faculty of Pharmacy in Hradec Králové,
Charles University, Czech Republic
[2] Maternal and Fetal Health Research Centre, Institute of Human Development, University of Manchester,
St. Mary's Hospital, Central Manchester University Hospitals NHS Foundation Trust, Manchester Academic
Health Science Centre, Manchester M13 9WL, UK
e-mail: neumz6aa@faf.cuni.cz

Current prevention of mother-to-child transmission of HIV infection is based on administration of combination antiretroviral therapy (cART) during the whole pregnancy. One

of the prophylactic mechanisms of cART is the presence of antiretrovirals in the fetal circulation that can be, nevertheless, associated with the potentially harmful effects on the developing fetus. To select optimal therapy while minimizing risks it is inevitable to have detailed knowledge of all the factors affecting transplacental transport of drugs.

The aim of this study was to investigate whether the main placental transporters are able to affect distribution of selected antiretrovirals between mother and fetus. Employing variety of *in vitro*, *in vivo*, *in situ* and *ex vivo* methods we determined the role of the drug efflux transporters in the transplacental pharmacokinetics of the tested drugs.

We suggested that antiretrovirals zidovudine, abacavir and tenofovir disoproxil fumarate are the substrates of placental ABCB1 and ABCG2 transporters. However, passive diffusion and/or other transporters enabled the penetration of abacavir and zidovudine into the fetus. On the other hand, the transplacental transport of lamivudine and parent drug tenofovir was not affected by the activity of ABC efflux transporters. Further we detected that long-term administration of tenofovir and emtricitabine to pregnant rats altered expression of the main drug efflux transporters in the selected organs of neither fetus nor mother.

The presented results contribute to the complex knowledge regarding transplacental pharmacokinetics of antiretroviral drugs.

The study was supported by Grant Agency of Charles University (SVV 2015/260-185).

RESPONSE OF SERUM INFLAMMATORY MEDIATORS TO CHEMOTHERAPY OF BREAST CANCER IN MICE AND HUMANS

ROZKYDALOVÁ, L.,[1] MIČUDA, S.,[2] TOMŠÍK, P.,[3] GRIM, J.,[4] ŠTAUD, F.[1]

[1] Department of Pharmacology and Toxicology, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[2] Department of Pharmacology, Faculty of Medicine in Hradec Králové, Charles University, Czech Republic
[3] Department of Medical Biochemistry, Faculty of Medicine in Hradec Králové, Charles University, Czech Republic
[4] Department of Oncology and Radiotherapy, Faculty of Medicine and Teaching Hospital, Hradec Králové, Charles University, Czech Republic
e-mail: rozkydal@faf.cuni.cz

Breast cancer (BC) is the most common cancer in women worldwide. Despite advances in early-stage diagnostics and new therapeutic approaches, BC still remains one of the most frequent causes of cancer mortality in women. One of the major factors behind pathophysiological complexity of BC and variability in response to chemotherapy seems to be reaction of immune system but data in this area are still sparse. The aim of study was therefore to evaluate changes in serum concentrations of cytokines accompanying chemotherapy in humans and mice with BC. Mice with Ehrlich BC tumor showed biphasic change in IL-1, IL-5, IL-6, IL-12, MCP1, VEGF, TNF, IP10/CXCL10, MIG/CXCL9, and KC/GRO1 where in comparison to control group, a decrease was seen at 5th day after tumor implantation, which was followed by marked increase at 10th day where tumor present in advanced stage. Chemotherapy with cisplatin led to almost complete disappearance of tumor and attenuation of immune response including IL-2 concentrations.

Doxorubicin showed only partial effect on tumor size and cytokine response. In comparison, neoadjuvant chemotherapy (NCT) in patients with BC produced reduction in IL-1, IL-2, cFGF, EGF, and MIP-1, but also increase in IP-10, INF, and IL-2R when compared with levels measured before initiation of NCT. In conclusion, our data showed marked difference between mice and humans with BC in systemic cytokine/chemokine response to administration of chemotherapy. On the other hand, similarity in both species was found in reaction of IL-1 and IL-2, which may suggest significant role of these two cytokines in BC pathophysiology, and therapeutic response.

The study was supported by IGA NT/13473-3/2012.

EFAVIRENZ DECREASES RENAL EXCRETION OF LAMIVUDINE THROUGH INHIBITION OF OCT1, OCT2 AND MATE1 DRUG TRANSPORTERS

ŘEZNÍČEK, J., ČEČKOVÁ, M., ŠTAUD, F.

Department of Pharmacology and Toxicology, Faculty of Pharmacy in Hradec Králové,
Charles University, Czech Republic,
e-mail:reznj7ba@faf.cuni.cz

Efavirenz (EFV) is a non-nucleoside reverse transcriptase inhibitor that constitutes an important part of combination antiretroviral therapy (cART) in HIV positive patients. It is often combined with other antiretrovirals, mainly nucleoside reverse transcriptase inhibitors (NRTI), many of which are substrates of solute uptake carriers OCTs (SLC22A) and efflux transporters MATEs (SLC47A). These membrane proteins are functionally expressed in kidneys and contribute to vectorial transfer of many drugs across tubular cells from blood to urine. Drug–drug interactions (DDI) on these transporters can affect renal drug excretion of the drug substrate and/or its accumulation in the renal tissue.[1] The aim of our study was to evaluate inhibitory potential of efavirenz towards OCT1, OCT2 and MATE1 transporters and possible transporter-mediated DDI between efavirenz and another NRTI used in cART, lamivudine, which has been previously shown to be actively excreted into urine by OCTs and MATE transporters.[2]

The inhibitory effect of efavirenz to OCT1, OCT2 or MATE1 transporters was measured *in vitro* using accumulation and transport assays in MDCK cell lines stably expressing the human SLC transporters. ASP^+ and MPP^+ were used as model substrates of OCT1, OCT2 and MATE1. Effect of efavirenz on pharmacokinetics of lamivudine was further evaluated in male Wistar rats *in vivo*.

Employing *in vitro* approaches, efavirenz was able to inhibit uptake of ASP^+ into relevant MDCK-OCT1, MDCK-OCT2 and MDCK-MATE1 cells with IC_{50} values of 4.2, 7.5 or 53.7 μM respectively. Efavirenz (10 μM) significantly decreased transcellular transport and intracellular accumulation of MPP^+ (2 nM) as well as lamivudine (10 nM) across cellular monolayers of MATE1 expressing cell lines. When applicated intravenously to Wistar rats, 10 μM efavirenz significantly decreased renal excretion of lamivudine by up to 92.13% and increased lamivudine accumulation in kidney 10.39 fold, exceeding thereby the effect of cimetidine as a control inhibitor of OCT and MATE transporters (causing inhibition of renal excretion by 55.73% and renal lamivudine retention enhanced 9.56 fold).

Taken together, our data suggest that efavirenz is an inhibitor of OCT1, OCT2 and MATE1 transporters able to cause pharmacokinetic DDI able to influence renal excretion of lamivudine *in vivo*. Further study will be needed to justify these findings in clinical pharmacotherapy.

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SOLUBLE ENDOGLIN EFFECTS ON ENDOTHELIAL CELLS *IN VITRO*

VAREJČKOVÁ, M., NĚMEČKOVÁ, I., FIKROVÁ, P., DOLEŽELOVÁ, E., VITVEROVÁ, B., NACHTIGAL, P.

Department of Biological and Medical Sciences, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
e-mail: varem7aa@faf.cuni.cz

Endoglin (Eng) is a transmembrane accessory type III receptor for the transforming growth factor-β (TGF-β) and its expression is up-regulated in proliferating endothelial cells. Soluble form of endoglin (sEng) has been identified in plasma of patients with preeclampsia, atherosclerosis and other cardiovascular diseases. Hence, endothelial dysfunction plays important role in many cardiovascular pathologies, we decided to test, whether high soluble endoglin induces changes in the expression of markers of endothelial dysfunction, inflammation, and oxidative stress in human umbilical vein endothelial cells (HUVEC) *in vitro*.

HUVEC were obtained from Lonza from Clonetics™ Laboratories. Cells were cultured in gelatin-coated flasks in EGM-2 medium. HUVEC were exposed to recombinant human sEng (50 ng/ml or 500 ng/ml) for 3 and 16 hours. qRT-PCR was used for the evaluation of changes in the expression of mRNA of selected markers.

The results of this study showed that treatment of HUVECs with 500 ng/ml recombinant human sEng for 16 hours induced significant increase in the expression of pro-inflammatory markers. Surprisingly, markers of endothelial function/dysfunction and oxidative stress were not significantly affected. Significantly increased expression of membrane endoglin mRNA might represent potentially compensative and possibly protective mechanism how endothelial cells react on soluble endoglin treatment. In conclusion, this study shows that high concentration of soluble endoglin affects endothelial cells with respect to inflammatory markers. However, these results deserve further study, particularly on the protein level.

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CAN ANTIDEPRESSANT PREMEDICATION REDUCE ISCHAEMIC-REPERFUSION INDUCED INJURY?

VICEN, M., GULAČ, P., STANKOVIČOVÁ, T.

Department of Pharmacology, Faculty of Pharmacy, Comenius University, Bratislava, Slovac Republic
e-mail: matej.vicen@gmail.com

In 2012 was ischaemic heart disease the leading cause of death in the world according to WHO. Antidepressants can affect heart function. Adverse effects of antidepressant drug therapy occure mostly at the beginning of therapy. More than 10% of drug intoxications in Slovakia during year 2013 were caused by antidepressants. Aim of work was to clarify if one application of antidepressants to rats (30 mg/kg s.c. of amitriptyline, citalopram or venlafaxine) 24 hours before experiment with isolated rat heart is sufficient to modify ventricular function during the reperfusion phase. Isolated hearts were perfused according to the Langendorff (20 min stabilization + 35min ischaemia and 45 min reperfusion). In I-R (ischaemic-reperfusion – premedicated by vehicle) group was lowest incidence of dysrhythmias during reperfusion phase. Premedication with amitriptyline induced the highest incidence of different VPB forms in the end of reperfusion. Hearts from this group reacted on the beginning of reperfusion with increased heart frequency, ventricular tachycardia and fibrillation and increase in bradycardia epizodes incidence and duration at later stages of reperfusion. Citalopram premedicated hearts reacted predominantly by bradycardia. The incidence of dysrhythmias in this group was the highest at the beginning of reperfusion. Hearts affected by venlafaxine reacted at the beginning of reperfusion by similar incidence of bradycardia and ventricular tachycardia. Incidence and duration of dysrhytmias were increased after antidepressant premedication. Incidence of dysrhytmias was in order: I-R < Citalopram < Venlafaxine < Amitryptyline group. Antidepressant premedication didn't reduce effect of I-R injury on the heart's function. Antidepressant premedication worsened function of hearts after ischaemia.

This work was supported by grants VEGA, SR 1/1342/12 and UK/430/2015.

BIOORGANIC AND PHARMACEUTICAL CHEMISTRY SECTION

INTRAMOLECULAR TSUJI-TROST ALLYLATION – ENANTIOSELECTIVITY OUTLOOK

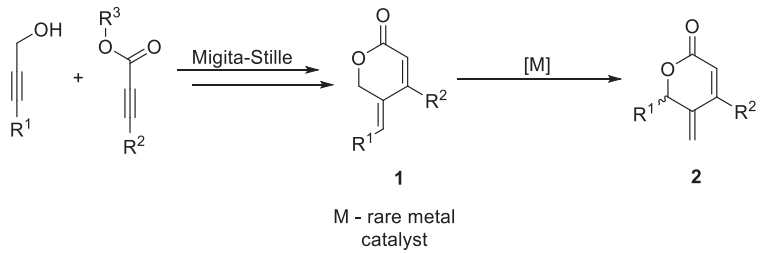
BRŮŽA, Z., KRATOCHVÍL, J., POUR, M.

Department of Inorganic and Organic Chemistry, Faculty of Pharmacy in Hradec Králové,
Charles University, Czech Republic
e-mail: bruzazb@faf.cuni.cz

Our group previously reported an interesting by-product of Migita-Stille coupling.[1] The original required products (**1**) underwent a rearrengement to isomeric 5,6-dihydro-

5-methylene-2*H*-pyran-2-ones (**2**). Plausible mechanism for this transformation has been proposed.

The rearrangement introduces a new chiral center to the pyranones. The newly formed compounds (**2**) may be further utilized as precursors to norditerpenoid structures through the reactivity of electrophilic Michael-acceptor sites and ester moiety reduction. Therefore we performed screening of catalysts, chiral ligands, solvents and additives to achieve the highest possible enantiomeric excess.



Scheme 1.

This work was supported by Grant Agency of Charles University (project No. 1176213), Czech Science Foundation (project No. 15-07332S) and Faculty of Pharmacy in Hradec Králové (SVV-260-183).

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DESING AND SYNTHESIS OF NOVEL 3,4-DIARYLSUBSTITUTED FURANONES FOR GROWTH-INHIBITORY AND PRO-APOPTOTIC EFFECT AGAINST LEUKEMIA CELLS

HORKÝ, P.,[1] VORÁČOVÁ, M.,[1] VACEK, J.,[2] POUR, M.[1]

[1] Department of Inorgancic and Organic Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[2] Department of Medical Chemistry and Biochemistry, Palacký University, Olomouc, Czech Republic
e-mail: horkyp@faf.cuni.cz

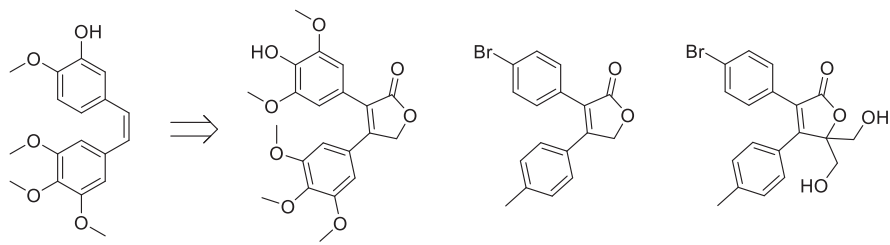
Herein we report the synthesis, derivatization and cytostatic activity evaluation of 3 libraries of α,β-diphenyl furanones.

The first library was derived from natural combretastatin. Since the *cis*-stilbene structural pattern as well as 3,4,5-trimethoxy substitution are essential for antitumor activity, the first library of molecules was characterized with high oxygenation of both phenyl rings.

In the second series of compounds, different substituents were attached. Furanones bearing halogen on C3 aromatic core and alkyl or alkoxy group on C4 found to possess sig-

nificant antineoplastic activity against human leukemia cancer cell lines. More specifically, several compounds proved to possess activity at the submicromolar range. Furthermore, the effect on healthy cell lines was also investigated. No toxicity observed up to a concentration of 40μM in medium.

In order to increase the hydrophilicity of our analogs a third library was developed by the introduction of two hydroxymethyl groups at the structure. Unfortunatelly, the activity of the obtained molecules was decreased.



Combretastatin A4 | Example of 1st library K562 (IC_{50} > 50 μM) | Example of 2nd library K562 (IC_{50} < 1 μM) | Example of 3rd library K562 (IC_{50} > 50 μM)

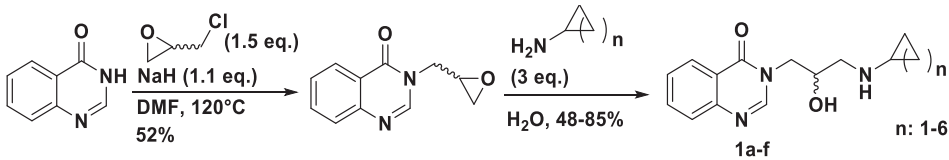
This work was supported by Charles University (GAUK 1906214, SVV 260-183) and Czech Science Foundation (15-073325).

NOVEL BIOLOGICALLY ACTIVE QUINAZOLINES

HRUŠKOVÁ, Z. R.,[1] BALMAGAMBETOV, D.,[1] VOPRŠÁLOVÁ, M.,[2] POUROVÁ, J.,[2] PÁVEK, P.,[2] ŠPULÁK, M.[1]

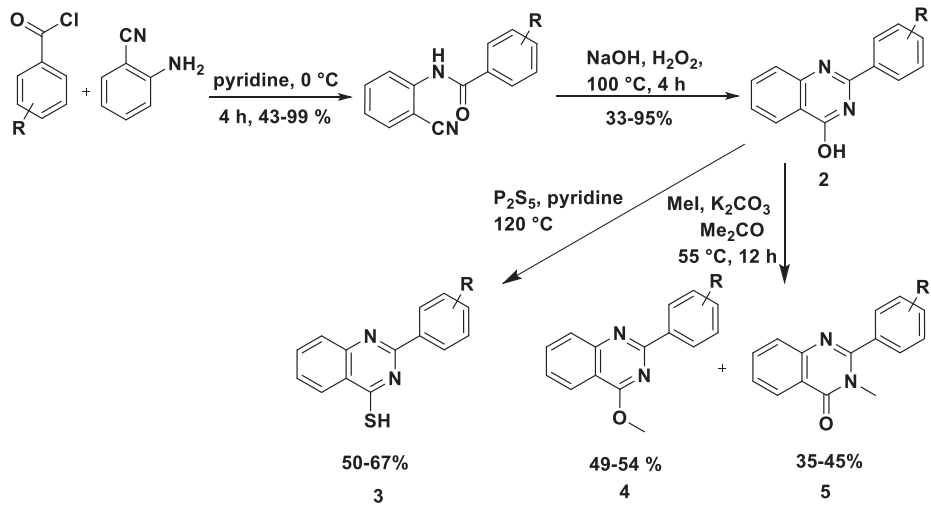
[1] Department of Inorganic and Organic Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[2] Department of Pharmacology and Toxicology, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
e-mail: hruskovz@faf.cuni.cz

The most active bronchodilatory compounds from previous screening contained (piperidine-1-yl)propyl moiety attached to quinazoline ring.[1,2] Another series of derivatives (**1a–f**) bearing hydroxyl group on the three-membered carbon linker were synthesized (Scheme 1). Bronchodilatory activity was tested and the relationship between the biological effect and the prepared compounds will be discussed.



Scheme 1.

As a result of random screening, 2-aryl-quinazoline-4-oles (**2**) have been found as potential ligands towards CAR receptor. We have synthesized a library of 65 sulfur (**3**), *O*-alkylated (**4**) and *N*-alkylated (**5**) analogues (Scheme 2).[3] The evaluation of affinity to CAR receptor displayed promising effects.



Scheme 2.

The study was supported by Charles University (GAUK 398315), SVV-260-183 and Czech Science Foundation (15-073322S).

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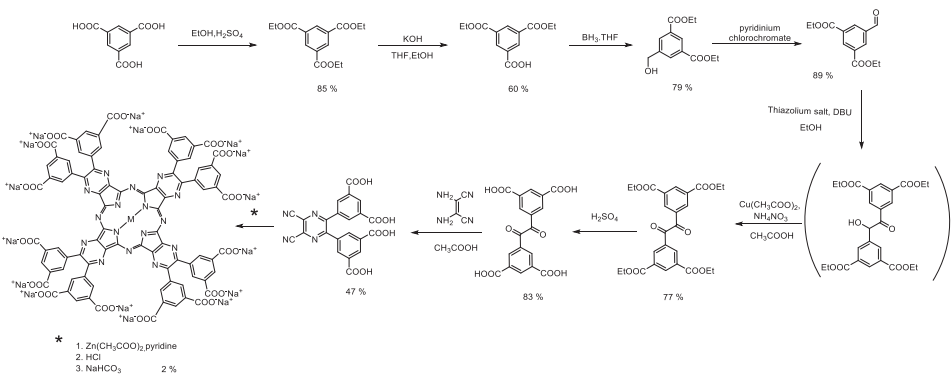
SYNTHESIS OF AZAPHTHALOCYANINE CONTAINING ANIONIC GROUPS

KOLLÁR, J., ZIMČÍK, P.

Department of Pharmaceutical Chemistry and Pharmaceutical Analysis, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
e-mail: kollarj@faf.cuni.cz

Phthalocyanines and their aza-analogues (e.g. tetrapyrazinoporphyrazines, TPyzPz) represent an interesting group of organic dyes with interesting photophysical properties (strong absorption in area over 650 nm and strong singlet oxygen production) highly suitable for the use in photodynamic therapy of cancer. The aim of this work was a synthesis

of a water-soluble sodium salt of zinc TPyzPz with eight 3,5-dicarboxylatophenyl substituents. Firstly, pyrazinedicarbonitrile precursor was prepared by multistep reaction pathway according to Scheme below. TPyzPz substituted with sixteen free carboxylic groups was synthesized in a template reaction with zinc(II)acetate in pyridine. This zinc(II) TPyzPz was converted into the sodium salt and the product was then purified by gel chromatography. Sixteen negative charges in rigid arrangement on periphery of the macrocycle inhibited aggregation in water or buffers of pH > 5.8. Strong aggregation was observed in buffers of pH < 4.8 due to the protonation of carboxylate functions and loss of repulsive forces. Final TPyzPz was tested on photodynamic activity *in vitro* on HeLa cells (IC_{50} = 5.7 ± 1.1 μM). Strong interactions with serum proteins and dependence of photodynamic activity on pH inside the cellular compartments was observed.[1]



The work was supported by SVV 260 183 and by Czech Science Foundation (13-27761S).

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SALICYLANILIDE-OLIGOTUFTSIN CONJUGATES: SYNTHESIS AND BIOLOGICAL ACTIVITY

KRÁTKÝ, M.,[1] BARANYAI, Z. S.,[2] BÖSZE, S. Z.,[2] VOSÁTKA, R.,[1] VINŠOVÁ, J.[1]

[1] Department of Inorganic and Organic Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[2] MTA-ELTE Research Group of Peptide Chemistry, Budapest, Hungary
e-mail: martin.kratky@faf.cuni.cz

The global tuberculosis epidemic and increasing emergence of drug-resistant tuberculous as well as atypical strains call for intensive research on new antimycobacterial agents, especially on new structures with innovative mechanisms of action and without cross-resistance.[1]

Salicylanilides (2-hydroxy-*N*-phenylbenzamides) have exhibited a significant *in vitro* activity against *Mycobacterium tuberculosis* including drug-resistant strains and atypical mycobacteria at low micromolar concentrations. However, they share pronounced cytotoxicity and poor solubility.[2] These obstacles can be overcome, *i.a.*, by employment of drug delivery systems. We selected peptide carriers based on repeated oligotuftsin sequence $[TKPKG]_n$, which are nontoxic, stimulate the immune system and target macrophages specifically.[3]

Oligotuftsin-based peptide carriers were obtained using solid-phase synthesis and Fmoc/tBu strategy. N-terminus and/or lysine side chain amino group(s) were modified by varied substituents (carboxylic acids, fluorescent labels, peptides etc.). Then, the carriers were coupled with convenient salicylanilide derivatives *via* oxime bond, purified and characterized.

The conjugates were evaluated for their *in vitro* extracellular antimycobacterial activity (two strains of *M. tuberculosis*, *M. abscessus*), intracellular activity in infected macrophages, cytotoxic and cytostatic properties for various cell lines, and cellular uptake. Salicylanilide-oligotuftsin conjugates showed improved activity and cellular uptake together with decreased toxicity.

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PERMEABILITY AND MICROSTRUCTURE OF MODEL LIPID MEMBRANES CONTAINING 6-HYDROXYCERAMIDES

KOVÁČIK, A.,[1] OPÁLKA, L.,[1] ŠILAROVÁ, M.,[1] MAIXNER, J.,[2] VÁVROVÁ, K.[1]

[1] Department of Inorganic and Organic Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[2] University of Chemistry and Technology in Prague, Czech Republic
e-mail: kovacika@faf.cuni.cz

Ceramides (Cer) based on 6-hydroxysphingosine (H) have been found only in human epidermis; however, their role in the skin barrier homeostasis is not fully understood. In this work we focused on the total synthesis of *N*-lignoceroyl-6-hydroxysphingosine (CerNH). Moreover, we aimed to study the permeability and microstructure of model lipid membranes based on CerNH in comparison with CerNS, CerNdS and CerNP. CerNH was prepared by using alkynylation of (*S*)-Garner aldehyde[1,2] with protected (*R*)-pentadec-1-yn-3-ol as a key step. Model membranes were composed of Cer/free fatty acids (C_{16}–C_{24})/cholesterol/cholesteryl sulfate. Their permeability was assessed in Franz-type diffusion cells using the following permeability markers: flux of two model compounds (theophylline and indomethacin), electrical impedance and water loss through the membrane. To elucidate the mechanisms of Cer effects on skin permeability, their biophysical properties were investigated by X-ray powder diffraction (XRPD) and infrared spectroscopy (ATR-FTIR). Using XRPD we found the short periodicity phase and crystalline

cholesterol in all membranes. In addition, in CerNH-based membrane we observed also a long periodicity phase with a repeat distance d = 10.6 nm. Next, using ATR-FTIR we showed differences in lipid mixing, packing and thermotropic phase behaviour. In membranes containing hydroxylated Cer, *i.e.* CerNP and CerNH, free fatty acids did not mix with the Cer chains.

The work was supported by the Czech Science Foundation (13-23891S) and Charles University (GAUK 1868214 and SVV 260183).

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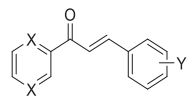
INHIBITION OF ALDOSE REDUCTASE BY CHALCONES: SYNTHESIS, ENZYME ASSAY AND MOLECULAR DOCKING

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

[1] Department of Pharmaceutical Chemistry and Pharmaceutical Analysis, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[2] Institute of Experimental Pharmacology and Toxicology, Slovak Academy of Science, Bratislava, Slovak Republic
e-mail: Marta.Kucerova@faf.cuni.cz

Aldose reductase (AKR1B1) is an important enzyme in polyol pathway, that is distinctly activated in metabolism of glucose under hyperglycemic conditions. Products of this pathway are implicated in long-term complications of diabetes.[1] In the aim of the search for new inhibitors, chalcone derivatives and its pyrazine analogues have been tested on isolated rat lens ALR2. Chalcones were prepared by Claisen-Schmidt condensation of acetofenone or pyrazin-2-ylethan-1-one with position isomers of hydroxybenzaldehyde or vanilline. In the enzyme assay, IC_{50} of the most active compounds ranged between 25–50 μM. In comparison with polyhydroxylated chalcones,[2] they exhibited lower activity. Interactions of the most potent chalcones in the active site of AKR1B1 were discussed in accordance with results of *in-silico* molecular docking.



X = CH, N
Y = 2-OH; 3-OH; 4-OH; 3-OCH_3,4-OH

The study was supported by PRVOUK P40 (Charles University) and by the project SVV 260 183.

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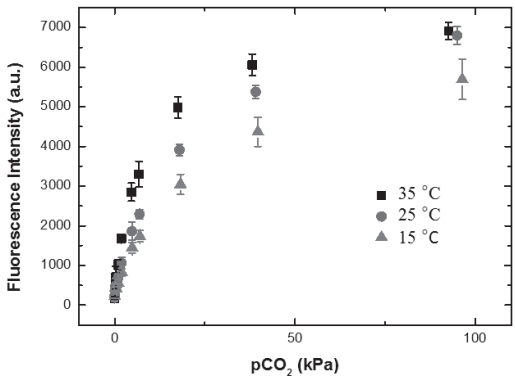
CO_2 SENSORS USING PHENOL SUBSTITUTED AZAPHTHALOCYANINES AS THE FLUORESCENT INDICATOR

LOCHMAN, L.,[1] NOVÁKOVÁ, V.,[1] ZIMČÍK, P.,[1] BORISOV, S. M.[2]

[1] Department of Pharmaceutical Chemistry and Pharmaceutical Analysis,
Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[2] Institute of Analytical Chemistry and Food Chemistry, Graz University of Technology, Graz, Austria
e-mail: lochmanl@faf.cuni.cz

Azaphthalocyanines (AzaPcs) are planar macrocyclic compounds with extensive system of double bonds. AzaPcs may be used as indicators for metal cations[1,2] or pH sensitive indicators.[3] The principle of their fluorescence sensing properties is based on blocking of intramolecular charge transfer upon analyte binding that leads to increase of fluorescence.

This project studies the possibility to use the pH sensitive AzaPcs as the fluorescence indicators in optical sensor devices for CO_2 detection. First, conditions for immobilization of AzaPc indicator into several matrices were optimized (e.g. type of hydrogel, solvent). Polyethylene terephthalate support foil was then coated by "cocktail" containing AzaPc indicator, matrice, solvent and base in thickness of the 75 μm of the wet film. Finally, prepared foils were characterized by spectral methods (absorption, emission) and sensitivity towards CO_2 was examined.



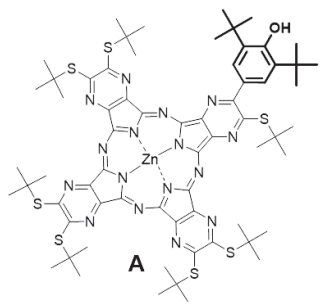


Fig. 1. Temperature dependency for compound **A** in Hydrothane 5 matrice

The study was supported by scholarship Aktion Austria-Czech Republic and SVV 260 183.

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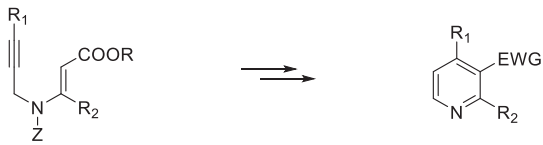
SYNTHESIS OF SUBSTITUTED PYRIDINES BY GOLD(I) CATALYSIS

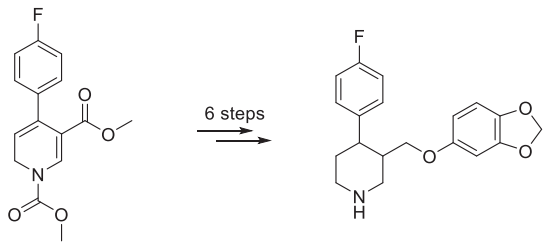
MATOUŠ, P., MIKUŠEK, J., JANOUŠEK, M., POUR, M.

Department of Inorganic and Organic Chemistry, Faculty of Pharmacy in Hradec Králové,
Charles University, Czech Republic
e-mail: matousp1@faf.cuni.cz

Synthesis of various types of heterocycles is possible from enyne precursors using cationic gold(I) species as a catalyst. In order to expand our research[1] on cyclisation of propargyl vinyl ethers to dihydropyrans using tris(2-furyl)phosphine gold(I) chloride (TFPAuCl) and silver tetrafluoroborate, we employed the same catalytic system on enynes with other heteroatoms. The synthetic protocol was optimized and a series of substituted nitrogen heterocycles synthesized. Crucial step of the synthesis is gold(I) catalysed endo-cyclization of 1,5-enynes. Compared with synthesis of pyrrols,[2] forming of dihydropyridnes was observed. The resultant dihydropyridine derivatives are potential precursors for paroxetine synthesis.[3]



R_1, R_2 = H, aryl, heteroaryl, alkyl
Z = Ts, MBS, Boc, -COOMe



This work was supported by Charles University (SVV 260 183 and GAUK 5671/2012) and Czech Science Foundation (Project No. 15-07332S).

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SYNTHESIS OF HAEMANTHAMINE DERIVATIVES

NĚMEČEK, J., HOŠTÁLKOVÁ, A., CHLEBEK, J., OPLETAL, L., CAHLÍKOVÁ, L.

Department of Pharmaceutical Botany and Ecology, Faculty of Pharmacy in Hradec Králové,
Charles University, Czech Republic
e-mail: nemej6aa@faf.cuni.cz

Isochinolines alkaloids are one of the brodest class of the secondary metabolites and with more than 2500 known structures constitute very large class of alkaloids with various biological properties.[1] Among these class of isochinolines alkaloides, the amarylidaceae alkaloids represent large group of natural compounds with antitumor, antibacterial, antifungal, antimalarial, antiviral, analgetic, acetylcholiesterase and butyrylcholinesterase inhibitory activity.[2]

Haemanthamine is one of the isochinolines alkaloid of the Amarylidaceae family. It is 5,10-β-ethanophenantridine derivative, which displayed pronounced cytotoxic activity againts various cancer cell line such as MOLT-4, HepG2, Hela, MCF7, CEM, K562, G-36, Bj human fibroblast, A 549, OE21, Hs683, U373, SKMEL and B16F10.[3]

In this work several derivatives of Haemanthamine were prepared and their inhibitory activities on acetylcholinesterase and butyrylcholineesterase as well as cytotoxic activity will be studied.

The study was supported by Charles University (SVV 260 184).

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SIGNALING ROLE OF CERAMIDES AND THEIR METABOLITES IN MAMMALIAN EPIDERMIS

OPÁLKA, L., VÁVROVÁ, K.

Department of Inorganic and Organic Chemistry, Faculty of Pharmacy in Hradec Králové,
Charles University, Czech Republic
e-mail: opall6aa@faf.cuni.cz

Ceramides (Cer) are essential lipids, participating in the formation of the mammalian epidermal barrier in the uppermost layer of the skin, the stratum corneum. As a minor component, they can be found in the cellular membranes. Cer and their metabolites (Cer-1-phosphate, sphingosine-1-phosphate, glycosphingolipids) are also well known to have a signaling role. They regulate proliferation, differentiation and apoptosis in epider-

mal keratinocytes and modulates innate immune function. The modulation in the signaling role of these molecules can be applied to cutaneous disease prevention and therapy.[1]

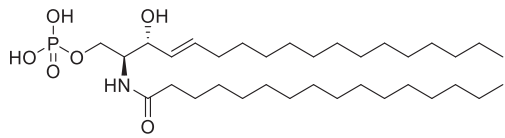

Fig. 1. Structure of Cer NS-1-phosphate.

This work focuses on the synthesis and evaluation of two Cer metabolites, Cer NS-1-phosphate and Cer NdS-1-phosphate. Few synthetic approaches have been published until now, but it's still a challenge to synthesize these compounds because of their low reactivity and high insolubility. For our synthesis, we decided to utilize a recently developed 3 steps synthesis, using a biphasic system for the introduction of the dimethyl phosphate group, followed by a hydrolysis to Cer-1-phosphate. The overall yield of this synthetic pathway is around 40% (even in the higher quantities, up to 100 mg).

The study was supported by the Czech Science Foundation (13-23891S) and by Charles University (SVV 260 183).

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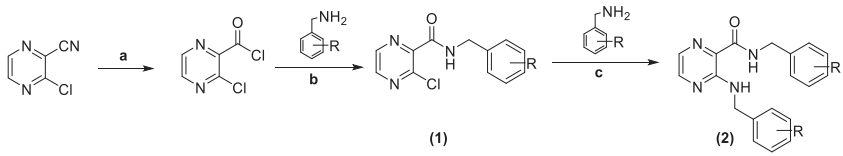
BENZYLDERIVATIVES OF 3-AMINOPYRAZINE-2-CARBOXAMIDE: SYNTHESIS AND ANTI-INFECTIVE EVALUATION

SEMELKOVÁ, L.,[1] ZÍTKO, J.,[1] JANĎOUREK, O.,[1] PATEROVÁ, P.,[2] KONEČNÁ, K.,[3] DOLEŽAL, M.[1]

[1] Department of Pharmaceutical Chemistry and Pharmaceutical Analysis, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[2] Department of Clinical Microbiology, University Hospital, Hradec Králové, Czech Republic
[3] Department of Biological and Medical Sciences, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
e-mail: semelkol@faf.cuni.cz

Pyrazinamide (PZA) is a first-line antitubercular drug and has been used for over sixty years. PZA has a significant role in shortening of tuberculosis treatment. As a small molecule PZA and metabolically derived pyrazinoic acid (POA) offer many ways how to affect mycobacteria: acidification of cytoplasm,[1] inhibition of trans-translation (liberation of ribosomes trapped in faulty protein synthesis),[2] inhibition of Fatty Acid Synthase I (synthesis of mycolic acids)[3] and aspartate dexarboxylase (involved in energetic metabolism).[4]

Series of substituted *N*-benzyl-3-chloropyrazine-2-carboxamides **(1)** and *N*-benzyl-3-(benzylamino)pyrazine-2-carboxamides **(2)** were prepared from the starting 3-chloropyrazine-2-carbonitrile *via* well known procedures.



Reagents and conditions:
a) 1. NaOH (aq); 2. acidification; 3. $SOCl_2$, DMF, PhMe, reflux
b) TEA, acetone, RT
c) pyridine, MeOH, microwave irradiation

R = 2-CH_3; 4-CH_3; 4-OCH_3; 2,4-OCH_3; 2-Cl; 3-Cl; 4-Cl; 2,4-Cl; 3,4-Cl; 2-F;4-F; 3-CF_3; 4-CF_3

Prepared compounds were characterized with analytical data and tested against four mycobacterial strains – *M. tuberculosis* H37Rv*, M. kansasii, M. avium* and *M. smegmatis*. Additionally, antibacterial and antifungal activity was determined. The most active compounds against *M. tuberculosis* (MIC = 12.5 μg mL^{-1}) were structures with double 3,4-dichlorobenzyl or 2-methylbenzyl substitution both in amide and amine moiety. Compund with 2-chlorobenzyl substitution in amide moiety showed the highest activity against *Streptococcus aureus* (MIC = 7.81 μmol L^{-1}). Structure-activity relationships within presented series and in comparison with previously published compounds will be presented.

The study was supported by the Grant Agency of Charles University, project B-CH/1594214, and SVV 260 183.

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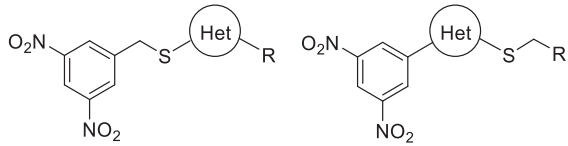
NITRO GROUP-CONTAINING HETEROAROMATIC COMPOUNDS AS ANTITUBERCULAR AGENTS: STRUCTURE-ACTIVITY RELATIONSHIPS STUDY

VALÁŠKOVÁ, L.,[1] ROH, J.,[1] KARABANOVICH, G.,[1] NĚMEČEK, J.,[1] STOLAŘÍKOVÁ, J.,[2] VÁVROVÁ, K.,[1] KLIMEŠOVÁ, V.,[1] HRABÁLEK, A.[1]

[1] Department of Inorganic and Organic Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[2] Laboratory for dg. of Mycobacteries, Department of Bacteriology and Mycology, Regional Institute of Public Heath, Ostrava, Czech Republic
e-mail: valaskl1@faf.cuni.cz

Tuberculosis (TB) is worldwide health and economic problem. Strains causing this infectious disease are becoming more and more resistant against therapy which has remained the same for more than 40 years. TB brings complication mostly in the underdeveloped world where higher incidence, poorer diagnosis and healthcare cause increased mortality and transmission of the infection as well as formation of resistant strains. A few totally drug resistant strains have appeared last years.

Our group has developed heterocyclic compounds containing 3,5-dinitrobenzyl/phenyl fragment (Figure 1). These compounds showed high activity against various mycobacterial strains, including resistant and dormant strains. Nitro groups are often carriers of increased toxicity, but not in case of our lead compounds. As a follow up to previous SAR study we examined the effects of various changes in 3,5-dinitrobenzyl/phenyl fragment to antimycobacterial activity.



Het = tetrazole, 1,3,4-oxadiazol
R = aryl, alkyl

Fig. 1.

This work was supported by the Charles University Grant Agency (project 361215/2015), Czech Science Foundation (project GAČR 14-08423S) and Charles University (project SVV 260 183).

SYNTHESIS OF TRICLOSAN DERIVATES AND THEIR ANTIMYCOBACTERIAL ACTIVITY

VOSÁTKA, R., KRÁTKÝ, M., VINŠOVÁ, J.

Department of Inorganic and Organic Chemistry, Faculty of Pharmacy in Hradec Králové,
Charles University, Czech Republic
e-mail: vosatkar@faf.cuni.cz

Development of novel tuberculosis chemotherapeutics against existing drug resistant strains is based on several strategies.[1] One of them involves the identification and inhibition of enzyme drug targets. *Mycobacterium tuberculosis* enoyl-acyl carrier protein reductase (InhA) important in fatty acid biosynthesis pathway, is a target of the frontline chemotherapeutic, isoniazid (INH). The majority of INH-resistant clinical isolates arise from mutations in KatG, the enzyme responsible for activation of INH, into its active form. Thus compounds that inhibit InhA without first requiring KatG activation could be active against the majority of INH-resistant strains of *M. tuberculosis*. Triclosan is well known inhibitor of InhA and a widely used broad-spectrum biocide.[2] Recent progress in the development of novel diphenyl ether-based InhA inhibitors[3] inspired us to prepare a new series of triclosan esters.

We have synthesized 28 triclosan esters based on various aliphatic, alicyclic, aromatic and heteroaromatic acids by the Steglich esterification or direct acylation with various acyl chlorides in presence of trimethylamine. Prepared derivatives were evaluated for their *in vitro* antimycobacterial activity against *M. tuberculosis* $H_{37}Rv$, *M. avium* and two strains

of *M. kansasii*. The best *in vitro* activity was found for 5-chloro-2-(2,4-dichlorophenoxy) phenyl 4-bromobenzoate with minimum inhibitory concentrations (MIC) of 16 μmol/L against *M. tuberculosis* $H_{37}Rv$. Triclosan ester of isonicotinic acid showed the best activity against atypical strains. Its MIC values was comparable with INH for *M. kansasii* 6509/96 and better for *M. avium* and *M. kansasii* 235/80 strains.

The study was supported by SVV 260 183.

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SECTION OF PHARMACOGNOSY AND TOXICOLOGY OF NATURAL PRODUCTS

ALKALOIDS FROM *NARCISSUS* CV. PROFESSOR EINSTEIN (*AMARYLLIDACEAE*) – ISOLATION AND BIOLOGICAL ACTIVITY

BREITEROVÁ, K.,[1] HOŠŤÁLKOVÁ, A.,[1] OPLETAL, L.,[1] KUNEŠ, J.,[2] CAHLÍKOVÁ, L.[1]

[1] Department of Pharmaceutical Botany and Ecology, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[2] Department of Inorganic and Organic Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
e-mail:breiterk@faf.cuni.cz

More than 500 *Amaryllidaceae* alkaloids (AmA) have been detected in plants of many different species belonging to the *Amaryllidaceae* family. These alkaloids showed wide range of biological activities. They are isolated from plant material and tested for their possible use in treatment of various illnesses. The most important AmA is galanthamin which is already used in the treatment of Alzheimer's disease as an inhibitor of human erythrocytic acetylcholinesterase (HuAChE; $IC_{50,HuAChE} = 1.5 \pm 0.2$ μM).[1] Some alkaloids display more biologic activities together. Active AmA serve as a template for a synthesis of series of semisynthetic analogues. Among the most widely used template AmA belong lycorine and haemanthamine.

From our previous screening on plants of *Amaryllidaceae* family, *Narcissus* cv. PROFESSOR EINSTEIN was chosen for detailed phytochemical work. Summary alkaloidal extract has been prepared from fresh bulbs (34.336 kg) and separated by column chromatography (Al_2O_3). Almost five hundred fractions were collected and, based on analytical TLC, pooled into 27 subfractions. Three substances were already obtained in crystallic form – isomer of hippeastrine, lycorine and haemanthamine, so far. Lycorine and haemanthamine have been already previously isolated at our department. They are currently used for the preparation of their semisynthetic analogues. Hippeastrine and other AmA which

are supposed, according GC/MS analysis, to be isolated in future will be screened for their biologic activity e. g. inhibition of HuAChE and HuBuChE (human butyrylcholinesterase), POP (prolyl oligopeptidase), GSK 3β (glycogen synthase kinase-3β), AKR1C3 (aldo-keto reductase 1C3) and others.

The study has been supported by SVV 260 184.

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ISOLATION OF ALKALOIDS FROM *NARCISSUS DUTCH MASTER* AND THEIR BIOLOGICAL ACTIVITIES

HULCOVÁ, D.,[1] CHLEBEK, J.,[1] OPLETAL, L.,[1] KUNEŠ, J.,[2] CAHLÍKOVÁ, L.[1]

[1] Department of Pharmaceutical Botany and Ecology, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[2] 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. Deficit of the neurotransmitter acetylcholine (ACh) in the cortex participates on the development of the AD, which results in the damage of cholinergic functions, and this is responsible for the memory loss. Acetylcholinesterase (AChE) is enzyme which hydrolyzes ACh and terminates process on nerve impulse transmission. Second important enzyme is butyrylcholinesterase (BuChE) which hydrolyzes ACh and another esters. The level of AChE decreases during AD but level of BuChE increases. This fact is the reason for looking at new substances with inhibition of activity against both cholinesterases. Today, inhibition of AChE is the most important goal in the treatment of AD.

Amaryllidaceae plant family is source for structurally unique compounds, Amaryllidaceae alkaloids. Some of these alkaloids show biological activities as anticancer, anticholinesterase and antiviral. For this cause the Amaryllidaceae family is interesting goal in searching for active substances with various biological activities. Galanthamine, an Amaryllidaceae alkaloid is already used as inhibitor of AChE in therapy of AD.

The summary ethanolic extract was prepared from the fresh bulbs of *Narcissus Dutch* Master L. More than three hundred fractions were collected by column chromatography (on Al_2O_3). Fractions were pooled into 16 subfractions. So far, seven pure alkaloids have been isolated. The isolated compounds were identified as homolycorine, seco-isopowellaminone, lycorenine, oduline, masonine, haemanthamine and tetrahydromasonine by comparison with the literature data and results of MS and NMR studies. The alkaloids are screened for their biological activities (the inhibition activity against HuAChE and HuBuChE, prolyl oligopeptidase (POP), glycogen synthase kinase-3β (GSK 3β), aldo-keto reductase 1C3 AKR1C3).

ANTIPLATELET EFFECTS OF FLAVONOIDS ON ARACHIDONIC ACID BASED PATHWAY

KARLÍČKOVÁ, J.,[1] ŘÍHA, M.,[2] FILIPSKÝ, T.,[2] MACÁKOVÁ, K.,[1] HRDINA, R.,[2] MLADĚNKA, P.[2]

[1] Department of Pharmaceutical Botany and Ecology, 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: karlickova@faf.cuni.cz

Flavonoids, common natural compounds of human diet, have a positive influence on various cardiovascular diseases according to experimental studies.[1] The aim of this study was to analyze anti-platelet activities of 29 flavonoids on three consecutive steps of the arachidonic acid cascade in detail. The isoflavonoids genistein and daidzein were shown to possess a marked cyclooxygenase-1 inhibitory activity, which was higher than that of acetylsalicylic acid using the isolated ovine enzyme, and physiologically relevant, although lower than in human platelets. Flavonoids with isolated 7-hydroxyl group and/or a 4′-hydroxyl group acted as antagonists on thromboxane A_2 (TXA_2) receptors. None of the tested flavonoids possessed an effect on TXA_2 synthase in a clinically achievable concentration.[2] An interesting finding is that the substitution of the free 7-hydroxyl group by a glucose did not block the activity. Several flavonoids inhibited two steps of the arachidonic acid pathway of platelet activation.

In conclusion, the consumption of flavonoids in food, particularly the isoflavonoids genistein and daidzein, may affect platelet aggregation.

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TRANSPORT MECHANISMS IN CELL MEMBRANE OF RED CLOVER

KUBEŠ, J., MARTIN, J., KAŠPAROVÁ, M., TŮMOVÁ, L.

Department of Pharmacognosy, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
kubej7aa@faf.cuni.cz

The red clover (*Trifolium pratense*) from family *Fabaceae* contains several groups of secondary metabolites, which are used in medicine. Isoflavonoids belong to a group of phytoestrogens, because they can bind to corresponding hormonal receptors and substitute or block their activity.[1] These secondary metabolites are produced inside plant cell and they are stored in a vacuole or transported to other parts of the plant. They can also be excreted outside to the soil; some isoflavonoids can attract nitrification bacteria, which are typical

for *Fabaceae* plants. The plant cell has several mechanisms as ABC or MATE transporters, which can be used for transport of these metabolites.[2] Transport studies were performed with suspension cultures of *T. pratense*, var. DO-8, which were derived from a callus. The cell suspension was treated with metavanadate and vanadyl sulphate for increasing of secondary metabolites at first. The highest content of secondary metabolites were found after application of these elicitors in concentration of 10 μmol L^{-1} and cultivation for 24 hours. The various inhibitors of transport were added then and their effect on secondary metabolites concentration was observed in medium and suspension. Inhibitor of proton pumps sodium orthovanadate and calcium channel blocker verapamil inhibited transport of some isoflavonoids as genistin. NH_4Cl and ABC inhibitor probenecid had no effect. The study will continue with more inhibitors as brefeldin, which can inhibit vesicular transport.[3]

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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] HOŠŤÁLKOVÁ, A.,[1] CHLEBEK, J.,[1] OPLETAL, L.[1]

[1] Department of Pharmaceutical Botany and Ecology, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[2] Department of Inorganic and Organic Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
e-mail: locarekm@faf.cuni.cz

Indole alkaloids are known as the source of cholinesterase inhibitors for the treatment of Alzheimer's disease.[1] 62 kg of dried aerial parts of *V. minor*, an ornamental plant from Apocynaceae family, were extracted in our laboratory. The mixture of the alkaloids (201 g) was chromatographed on alumina (total 531 fractions, combined in 16 fractions). The fractions 73–110, 128–146 and 147–214 were further separated using combination of flash chromatography and preparative TLC on silica-gel.

Two compounds were isolated from the fraction 73–110 and identified by GCMS and NMR as vincaminorine and vincaminorein.[2] Alkaloids (+)-minovincine, (+)-eburnamonine and vincorine were isolated from the fraction 128-146. Their structures were determined by NMR. Potentially new alkaloid named kosimonine was isolated from fraction 147-214. On its spectral characteristics (NMR, MS) and physical features (optical rotatory) is working presently.

All isolated alkaloids were tested for their inhibition activity on HuAChE and HuBuChE by Ellman's method.[3] Alkaloids exerted significant inhibitory of HuBuChE; the most potent inhibitors were vinkaminorein and vincorine with IC_{50} values of 71 ± 0.49 and 9.75 ± 0.45 μM respectively.

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PATHOBIOCHEMISTRY AND XENOBIOCHEMISTRY SECTION

SELECTED SESQUITERPENES FROM *MYRICA RUBRA* ESSENTIAL OIL POTENTIATED ACCUMULATION AND EFFICACY OF DOXORUBICIN IN CANCER CELLS.

AMBROŽ, M.,[1] ŠKARKA, A.,[2] SKÁLOVÁ, L.[1]

[1] Department of Biochemical Sciences, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[2] Department of Chemistry, Faculty of Science, University of Hradec Králové, Czech Republic
e-mail: ambrozm@faf.cuni.cz

Our previous experiments have shown that *Myrica rubra* essential oil (MEO) inhibited proliferation of Caco 2 cancer cells. Selected sesquiterpenes, α-humulene (HUM), caryophyllene oxid (CAO), *trans*-nerolidol (NER) and valencene (VAL), form significant proportion of MEO.[1] All listed sesquiterpenes alone have ability to decrease cancer cell proliferation in concentration dependent manner with IC_{50} in range 24–57 μg/mL. To investigate possible enhancement of efficacy of doxorubicin (DOX), frequently used cytostatic, we tested combinations of DOX with HUM, CAO, VAL and NER. CalcuSyn software was used for distinction of synergism, antagonism and additive effect as it was described by Chou.[2] Based on the obtained results, CAO, NER and VAL seem to synergistically increase effect of DOX, while HUM has only additive effect. DCF assay showed ability of HUM, CAO, VAL to increase production of ROS. On the contrary, NER decreased ROS production in cancer cells. All of the listed sesquiterpenes increased accumulation of DOX in Caco-2 cells, with CAO being the most effective.

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EXPRESSION PROFILE OF DHRS1 IN HUMAN TISSUES

ANDREJS, J.,[1] MALČEKOVÁ, B.,[1] LUNDOVÁ, T.,[1] ŠTAMBERGOVÁ, H.,[1] ZEMANOVÁ, L.,[1] ŠAFR, M.,[2] WSÓL, V.[1]

[1] Department of Biochemical Sciences, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[2] Institute of Legal Medicine, Faculty of Medicine, Charles University and University Hospital in Hradec Králové, Czech Republic
e-mail: Andrejsj@faf.cuni.cz

Dehydrogenase/Reductase Member 1 (DHRS1) belongs to superfamily of short-chain dehydrogenases/reductases (SDR). Wide variety of endogenous and xenobiotic substrates are metabolised by some members of SDR superfamily. However from group of 75 human genes, only 20% of its members are regarded as well-characterized. DHRS enzymes are group of 17 enzymes from SDR superfamily that are generally poorly characterized, but there is first knowledge of a role of some members (e.g. DHRS4, DHRS7)[1] in metabolism of some xenobiotic or endogenous substrates.

The aim of this study is determination of expression pattern of DHRS1 in normal human tissues collected in short post-mortem interval. RNA was isolated from tissues and its integrity was checked by 3′:5′ assay.[2] DHRS1 on mRNA expression level was measured by real-time reverse transcription polymerase chain reaction with absolute quantification in 15 tissues collected from 4 males. The expression pattern on protein level was measured by western blot in whole tissue homogenates. Highest expression of DHRS1 mRNA was detected in liver and lower expression was also detected in thyroid, testis, kidney and adrenals, but expression on protein level was detected only in liver. These results represents part of effort to characterize previously unknown enzymes and tissue expression levels together with their *in vitro* activity may suggest their potential role in human organism.

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FIRST IDENTIFICATION OF DHRS1 EZYME AS SPECIFIC MOLECULAR TARGET OF ANTICANCER DRUG ORACIN

ANDRÝS, R.,[1] ZEMANOVÁ, L.,[1] LENČO, J.,[1,2] WSÓL, V.[1]

[1] Department of Biochemical Sciences, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[2] Institute of Molecular Pathology, Faculty of Military Health Sciences, University of Defence, Brno, Czech Republic
e-mail: andrysr@faf.cuni.cz

For several millennia the human medicine is based on application of small bioactive molecules that are administered in the form of plant extracts or synthetic compounds. However, their use in modern medicine is not possible without a detailed understanding of their biochemical effects and identification of their molecular targets. Chemical proteomics based on the specific recognition between the bioactive molecule and the target molecule is currently the most widely used techniques for identification of molecular targets of small molecules.[1] Carbonyl-reducing enzymes, which play an important role in physiology due to their involvement in metabolism of various endogenous (e.g. prostaglandins, steroid hormones) and xenobiotic (e.g. anthracyclines, oracin) substrates, also represent such target biomolecules. Although the majority of today known carbonyl-reducing enzymes represent soluble proteins, there are many membrane-bound members in short chain dehydrogenases/reductases (SDR) superfamily. However, the knowledge on their role in metabolism of xenobiotics is quite poor. Based on the research on the reduction stereospecificity of anticancer drug oracin, there were predicted microsomal carbonyl-reducing enzymes involved in the metabolism and inactivation. However, previous attempts to purify these enzyme failed.[2]

The aim of this project was to develop suitable affinity carrier with immobilized ligand oracin capable to selectively purify carbonyl-reducing enzymes from human liver tissue and subsequently determine the potential role of theses enzymes in biotransformation.[3] Thus, affinity carrier was implemented into purification protocol of human microsomal carbonyl-reducing enzymes. Obtained fractions exhibited metabolic activity towards oracin with desired stereospecificity of its reduction. Using mass spectrometry proteins DHRS1, RDH16 and 17β-HSD6, with unknown affinity and metabolic activity towards oracin were successfully isolated and identified. Furthermore, enzyme 11β-HSD1 with already described affinity towards oracin was identified too. The selectivity of enzyme DHRS1 towards oracin was subsequently demonstrated by incubation of recombinant protein with affinity carrier and by Drug Affinity Responsive Target Stability (DARTS) method. The confirmation of DHRS1 specific interaction with this drug may indicate its potential role in biotransformation of other xenobiotics.

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CYCLIN DEPENDENT KINASE INHIBITORS AS MODULATORS AND SUBSTRATES OF ABC TRANSPORTERS

ČÍHALOVÁ, D.,[1] ČEČKOVÁ, M.,[1] ŠORF, A.,[1] KUČERA, R.,[2] KLIMEŠ, J.,[2] ŠTAUD, F.[1]

[1] Department of Pharmacology and Toxicology, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[2] Department of Pharmaceutical Chemistry and Pharmaceutical Analysis, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
e-mail: cihad6aa@faf.cuni.cz

Cyclin-dependent kinases play an important role in cell cycle regulation and their enhanced activity can lead to the development of various malignancies. Therefore, these kinases have become a rational target for inhibition in cancer therapy. ABC efflux transporters influence the pharmacokinetic properties of various drugs and their overexpression in cancer cells lead to multidrug resistance (MDR) against diverse cytotoxic therapeutics. Three members of the ABC transporter family play a prominent role in the pharmacokinetics and MDR: ABCB1 (P-glycoprotein), ABCG2 (breast cancer resistance protein) and ABCC1 (multidrug resistance-associated protein 1).

The aim of this work was to elucidate the interactions of novel CDKIs, dinaciclib and palbociclib, with ABC transporters using *in vitro* methods. Moreover, we aimed to determine whether these interactions affect the efficiency of conventionally administered anticancer drugs in human cancer cells.

Applying the MDCKII cell model and monolayer transport assays, we identified dinaciclib as an inhibitor of ABCC1, but at the same time a substrate of ABCB1 and ABCG2 transporters. Using the accumulation method in MDCKII cell lines overexpressing ABC efflux transporters we revealed inhibitory properties of palbociclib towards ABCB1 and ABCG2 and synergistic antiproliferative effect of both CDKIs when applied in combination with conventional cytotoxic drugs that are substrates of ABC transporters. Our data thereby show the ability of studied CDKIs to interact with ABC transporters as inhibitors and/or substrates and suggest that transporter-mediated drug-drug interactions (DDIs) should be taken into account, when introducing the CDKIs into anticancer pharmacotherapy. The DDI of CDKIS with conventional cytotoxic drugs caused by the inhibitory activity of CDKIs towards the ABC transporters can be exploited to battle the problem of MDR.

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NEWLY SYNTHESIZED ANALOGUES OF DEXRAZOXANE – INITIAL *IN VITRO* CHARACTERIZATION

JIRKOVSKÁ, A.,[1] KARABANOVICH, G.,[2] ROH, J.,[2] GAVUROVÁ, L.,[1] EISNER, T.,[2] ŠUS, J.,[2] KRATOCHVÍL, M.,[2] VÁVROVÁ, K.,[2] ŠIMŮNEK, T.,[1] ŠTĚRBA, M.[1]

[1] Department of Biochemical Sciences, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[2] Department of Inorganic and Organic Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
e-mail: anna.jirkovska@faf.cuni.cz

Anthracyclines (ANT) belong to the most frequently used antineoplastic agents. However, their cardiotoxic effect is still within the dose-limiting factors in clinical practice. Although known for decades, the mechanisms of ANT cardiotoxicity are still elusive. The only clinically available protective compound against anthracycline cardiotoxicity is dexrazoxane (DEX). Nevertheless, DEX use in the clinical practise is limited, mainly due to the anticipated risk of antagonism in the antineoplastic effects of DEX and ANT or possible adverse effects of DEX. These hurdles could be minimized by rational design of DEX analogues. Unfortunately, the precise mechanism of DEX cardioprotection or structure-activity relationship of DEX or other bis-dioxopiperazines have not been fully resolved. Although the traditional hypothesis presumes DEX metabolism to iron-chelating EDTA analogue ADR-925 with subsequent protection from the reactive oxygen species formation, the role of oxidative damage in ANT cardiotoxicity has been critically reviewed and is widely discussed. Both ANT and DEX also interact with topoisomerase II, which is present not only in cancer cells, but also in cardiomyocytes. Therefore, the aim of this study is the synthesis of structural analogues of DEX with changes to both the piperazine rings and connective chain with subsequent study of their biological activity. Currently, 20 new analogues have been synthesized and evaluated for their antiproliferative activity using human promyelocytic leukaemia cell line HL-60 and cardioprotection in the *in vitro* model of ANT cardiotoxicity on isolated neonatal rat cardiomyocytes, several analogues have been studied also for their ability to chelate free catalytically active iron ions and also for their ability to inhibit the catalytic activity of topoisomerase II or modulate its content in isolated neonatal cardiomyocytes.

The study was supported by the Czech Science Foundation (13-15008S).

STUDY OF EXTRACELLULAR VESICLES PRODUCTION AND THEIR ROLE IN PATHOGENESIS OF *CANDIDA ALBICANS* INFECTION

KONEČNÁ, K.,[1] VEJSOVÁ, M.,[1] JÍLEK, P.,[1] KLIMENTOVÁ, J.,[2] KROCOVÁ, Z.,[2] BENADA, O.[3]

[1] Department of Biological and Medical Sciences, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[2] Department of Molecular Pathology, Faculty of Military Health Sciences, Hradec Králové, University of Defence, Brno, Czech Republic
[3] Institute of Microbiology ASCR, Laboratory of Molecular Structure Characterization, Prague, Czech Republic
e-mail: konecna@faf.cuni.cz

Candida albicans (C. albicans) is a polymorphic fungus causing infections that range from superficial infections of the skin to life-threatening systemic infections. Although several virulence factors have recently been revealed, further investigations on molecular basis for the purpose of understanding the pathogenicity mechanism of *C. albicans* are required.

Within the last decade, special attention is paid to a new secretion system in gram-negative bacteria consisting in outer membrane vesicles secretion. This transport mechanism allows releasing molecules with various biological functions into host environment. These may include molecules carrying key functions for pathogenesis of microbial infections. In case of pathogenic yeasts, only the extracellular vesicles of one pathogenic yeast, *Cryptococcus neoformans,* were revealed and analyzed until now.

C. albicans yeasts (model strain, clinical isolates) were cultivated in Sabouraud broth (optimal nutrition composition) and in chemically defined minimal growth medium supplemented with limited amount of glucose (nutrition stress induction). *C. albicans* were cultivated into late exponential growth phase in 37 °C with gentle shaking. Concentrated sterile filtrates obtained after cultivation of different *C. albicans* strains in different conditions were ultracentrifuged and acquired pellets were submitted to observation *via* flurescent microscopy and tranmission electron microscopy. Preparation of samples for MS analysis and identification of protein cargo of *C. albicans* putative extracellular vesicles is now in process.

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BETA-NAPHTHOFLAVONE INDUCTION: ACTIVITIES, PROTEIN AND RNA LEVELS OF DRUG METABOLIZING ENZYMES

LNĚNIČKOVÁ, K., MATOUŠKOVÁ, P., SKÁLOVÁ, L., SZOTÁKOVÁ, B.

Department of Biochemical Sciences, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
e-mail: lnenickk@faf.cuni.cz

Aryl hydrocarbon receptor (AhR) is a ligand-activated transcriptional factor and belongs to the family of proteins that mediates a variety of biological responses to environmental pollutants. AhR activates several metabolic and detoxification pathways, among

other xenobiotic metabolizing enzymes (XME). In our study, we examined time dependence of response of the mRNA, protein levels and activity of cytochrome P450 isoforms 1A1/2 (CYP1A1/2), glutathione S-transferase alpha (GSTA) and NAD(P)H:quinone oxidoreductase 1 (NQO1) to inducer and their correlation. To achieve this objective, the rat hepatocytes were exposed to β-naphthoflavone (BNF), one of the inducers of drug-metabolizing enzymes acting via AhR.

Rat hepatocytes were incubated with BNF for 2, 4, 12 and 24 hours. Activity, protein and mRNA levels of CYP1A1/2 were in good accordance and they continuously rise for 24 hours. Gradual increase was observed in the activity of NQO1 for 24 hours, while protein content was almost the same and mRNA level was highest two hours after treatment and it was slowly reduced to the control level during 24 hours. Activity of GSTA in BNF treated hepatocytes increased from 65% (4 hours) to 76% of controls during 24 hours. The level of GSTA mRNA in BNF treated hepatocytes was significantly increased two hours after treatment. During the rest of the experiment, determined levels of GSTA mRNA were close to levels in control hepatocytes. Protein levels of GSTA in treated cells are similar to the control ones throughout the whole experiment.

Taken together, each enzyme showed different response and correlation between levels of protein and mRNA expression and activity after BNF treatment, although they all are regulated via AhR.

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IN VITRO EVALUATION OF NON-AGREGATING (AZA)PHTHALOCYANINES – INFLUENCE OF PERIPHERAL SUBSTITUTION ON PHOTODYNAMIC ACTIVITY

MACHÁČEK, M., JEDLIČKOVÁ, A., FIRKOVÁ, P., PODHORSKÁ, G., PŮLKRÁBKOVÁ, M., KOLLÁR, J., NOVÁKOVÁ, V., ZIMČÍK, P., ŠIMŮNEK, T.

Department of Biochemical Sciences, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
e-mail: machamil@faf.cuni.cz

Photodynamic Therapy (PDT) and Vascular-Targeted Photodynamic Therapy (VTP) are two similar ways to treat localized non metastatic solid tumors. Both are using the same fundamental elements – photosensitizer (PS), molecular oxygen and visible red light. In PDT, PSs require time to be taken up by tumor cells before the irradiation. Red light, that has the highest permeability in tissues, is absorbed by PS. From the excited state, PS relaxes back to the ground state by emitting a photon (i.e. *via* fluorescence) or forming reactive oxygen species (ROS). The major pathway of cell demise depends mostly on the structure of PS and its subcellular localization. In PDT, subcellular structures of cancer cells are the primary targets of generated ROS. In VTP light activation starts while PS is localized in tumor vasculature causing damage of endothelium surface that leads to vascular injury and deprivation of nutrients and oxygen to the tumor. PSs involved in this study are

highly hydrophilic nonaggregating phthalocyanines[1,2] and azaphthalocyanines,[1,2,3] with absorption in near infra-red area (around 780 nm). Their photodynamic effect was studied on several cell lines – human cervical carcinoma (HeLa), melanoma (SK MEL 28), brest adenocarcinoma (MCF-7), lung adenocarcinoma (A549) in PDT or human immortalized endothelial cell line (EA.Hy926), human primary endothelial cells (HUVEC) and HeLa in VTP protocol respectively. Dark toxicity (without activating light) was also established on HeLa, EA.hy926, HUVEC and 3T3 (mouse nonmalignant fibroblast) cells. All the examined compounds were efficient PSs after irradiation with red light ($\lambda > 570$ nm, 12.4 mW/cm^2, 11.2 J/cm^2) reaching exceptionaly high photodynamic activity up to nanomolar concentrations while showing low inherent toxicity in the absence of light to both malignant and nonmalignant cells. PSs damage lysosomal (PDT) or plasma (VTP) membranes and are spread throughout the cell where they cause additional damage to other organelles. This leads to quick and efficient cell demise.

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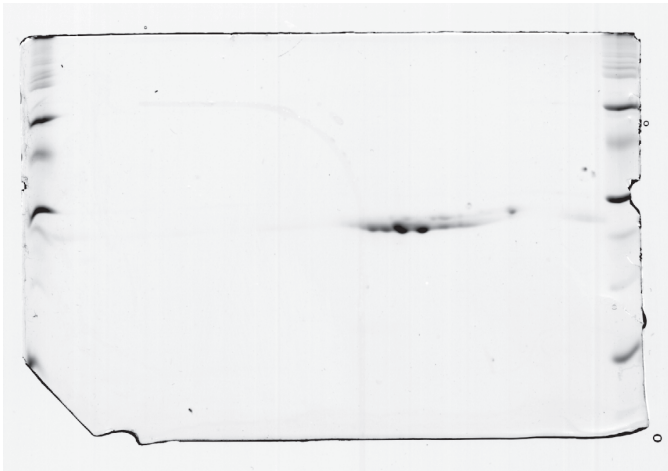
GLUTATHIONE S-TRANSFERASE IN RUMEN FLUKE *CALICOPHORON DAUBNEYI*

PRCHAL, L.,[1] BROPHY, P.,[2] MORPHEW, R.,[2] SZOTÁKOVÁ, B.[1]

[1] Department of Biochemical Sciences, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[2] IBERS, Aberystwyth University, Penglais, Aberystwyth, Ceredigion, United Kingdom, SY23 3FL
e-mail: prchall@faf.cuni.cz

Flukes are diverse group of parasitic helminths, that live in various parts of animal or human bodies and causing harm to animals and considerable economic losses.[1] *Calicophoron daubneyi* is rumen fluke parasiting in gastrointestinal tract of various ruminants, e.g. sheep and cattle. Small numbers of adult parasites cause little or no damage but severe damage of the intestine is caused by juvenile flukes traveling through digestive system and can be dangerous in case of weakened animals or heavy parasite burdens. Although its danger is not so grave as in the case of liver flukes the prevalence of rumen flukes has increased in previous years.[2] Our research was focused on glutathione S-transferases (GST), group of common eu- and xenobiotic metabolizing enzymes.[3] GST is also posible candidate for future vacines against flukes.[4] First extracts were prepared from rumen flukes, purified and activities assesed, then the samples were analyzed *via* 2D electrophoresis with coomassie staining, imunodetection and finally spots were analyzed by mass spectrometer.

It was found that GST activities in extract (cytosol-like) were about 0.7 µmol/min/mg and specific activity was increased with purification about 10 times. Glutathione agarose was more effective in GST purification than S-hexyl glutathione agarose. Several spots reacted with anti-Mu-GST and anti-Sigma-GST antibodies from other flukes. Finally the MS experiment confirmed similarities to the Sigma class GST of liver fluke *Fasciola hepatica.*



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IRON METABOLISM OF THE TUMOUR-INITIATING CELLS

RYCHTARČÍKOVÁ, Z.,[1,3] LETTLOVÁ, S.,[2,3] TRUKSA, J.,[2,3] VINŠOVÁ, J.[1,3]

[1] Department of Inorganic and Organic Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[2] Department of Genetics and Microbiology, Faculty of Science, Charles University, Czech Republic
[3] Laboratory of Tumour Resistance, Institute of Biotechnology, Czech Academy of Science, Prague, Czech Republic
e-mail: rychtarcikovazuzana245@gmail.com

An important role of iron in cancer growth and progression has been documented by studies reporting higher iron uptake due to altered metabolism in cancer cells. The con-

cept of tumour-initianting cells (TICs), also known as cancer stem cells (CSCc), claims that only a small portion of these cells is able to initiate the tumour growth and cause the formation of secondary tumours. The presence of TICs within tumour might be the reason for the failure of many conventional cancer therapies because of their higher resistance to anti-cancer drugs and/or apoptotic stimuli. It is known that there is higher iron requirement in proliferating cancer cells compared to normal ones and targeting iron metabolism with iron chelators can lead to apoptosis and cancer cell death, while supplemeting iron can block induction and promote their growth, but there are no data concerning TICs.

In our study we are using several specific culture conditions which enable cancer cell lines to grow in spheres exhibiting of 'stemness' markers – ABCG2, CD44, CDH2 and CD133. Our preliminary data show considerable differences in iron metabolism and in the expression of genes related to iron metabolism in TICs for example *ACO1, GLRX5, TFRC*, *QSOX1* and *TMPRSS6*.

We have generated genetically modified cell lines that can inducibly overexpress these genes upon doxycycline addition. This provide us with a tool to define the role of differentially expressed iron metabolism-related genes on cellular proliferation, migration and contribution to stem cell phenotype. Furthermore, the most significantly altered genes will be tested also *in vivo* model by using xenotransplantation in nude mice.

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RIBOCICLIB AND AZD5438 MODULATE ABC TRANSPORTER-MEDIATED MULTIDRUG RESISTANCE *IN VITRO*

ŠORF, A., ČÍHALOVÁ., D., ČEČKOVÁ, M., ŠTAUD, F.

Department of Pharmacology and Toxicology, Faculty of Pharmacy in Hradec Králové,
Charles University, Czech Republic
e-mail: sorfal@faf.cuni.cz

Drug transporters from the ATP-binding cassette (ABC) family actively efflux wide range of substrates including drugs out of the cells diminishing thereby their intracellular concentration under cytotoxic levels. Overexpression of ABC transporter members ABCB1, ABCG2 and ABCC1 leads to development of multidrug resistance (MDR) in cancer cells resulting in treatment failure.[1] The aim of our study was to characterize the ability of ribociclib and AZD5438, the novel anticancer cyclin-dependent kinase inhibitors (CDKi) to modulate MDR caused by ABCB1-, ABCG2- and ABCC1-mediated efflux.

In order to investigate the inhibitory potential of CDKi to the ABC transporters, we performed Hoechst 33342 and daunorubicin (DNR) accumulation assays in MDCKII cells stabily overexpressing ABCB1, ABCG2 and ABCC1. Subsequently, we evaluated whether the studied CDKi affect sensitivity of overexpressing cells to the anticancer substrates DNR and mitoxantrone (MIT) by employing XTT proliferation assay.

Our accumulation studies revealed AZD5438 as inhibitor of all three studied ABC transporters, with highest inhibitory potency to ABCC1. Ribociclib was shown as inhibitor

of ABCB1 and ABCG2, not affecting ABCC1 activity. Proliferation assays further demonstrated that ribociclib possesses the ability to sensitize ABCB1 and ABCG2 overexpressing cells to DNR and MIT, respectively, while AZD5438 potentiated DNR cytotoxic properties in MDCKII-ABCC1 cells. We therefore confirm the studied CDKi as modulators of *in vitro* ABC transporter-mediated MDR.

The study was supported by GAUK 344315/C/2015 and by SVV/2015/260-185.

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A PROMISING ROLE OF HUMAN DHRS7 IN THE METABOLISM OF RETINOIDS AND STEROIDS?

ŠTAMBERGOVÁ, H.,[1] ZEMANOVÁ, L.,[1] LUNDOVÁ, T.,[1] MALČEKOVÁ, B.,[1] ŠAFR, M.,[2] WSÓL, V.[1]

[1] Department of Biochemical Sciences, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[2] Institute of Legal Medicine, Faculty of Medicine in Hradec Králové, Charles University, Czech Republic
e-mail: hana.stambergova@faf.cuni.cz

In humans, 75 short-chain dehydrogenases/reductases (SDR) have been identified, so far. Some of them have received considerable attention because of their crucial role in the metabolism of various compounds such as steroids and retinoids that are involved in a broad spectrum of physiological processes. However, significant portion of SDR members remains without any assignment of function.

Dehydrogenase/reductase (SDR family) member 7 (DHRS7) belongs among such poorly characterized SDR proteins belongs. The enzyme is a member of cluster 3 of "classical" SDR; such members are considered to be retinoid and steroid metabolizing enzymes. The aim of this study is to provide evidence for DHRS7 to be a player in the metabolism of these signalling molecules. Recently, we determined DHRS7 to be an integral membrane protein of the endoplasmic reticulum facing the lumen which has shown at least *in vitro* NADPH-dependent reducing activity toward physiologically important substrates such as androstene-3,17-dione, cortisone and all-*trans*-retinal as well as several xenobiotics bearing a carbonyl moiety. Expression patterns of DHRS7 at the mRNA as well as protein level were evaluated in a panel of various human tissue samples. DHRS7 is expressed in tissues such as prostate, adrenal glands, liver or intestine, which could correspond with proved *in vitro* catalytic activities. These results will lay the foundation for an understanding of DHRS7 role in human (patho)physiology.

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SECTION OF CLINICAL AND SOCIAL PHARMACY

STORE MEDICAMENTS AND PHARMACEUTICAL INGREDIENTS IN GHETTO TEREZÍN DURING SECOND WORLD WAR

ARNDT, T.

Department of Clinical and Social Pharmacy, Faculty of Pharmacy in Hradec Králové,
Charles University, Czech Republic
e-mail: arndtt@faf.cuni.cz

Theresienstadt Ghetto, also known as Theresienstadt concentration camp was established by the Nazis in n the fortress and garrison city of Terezín. More than 150,000 other persons were held in Theresienstadt before being seen sent by rail transports at Treblinka and Auschwitz extermination camps in occupied Poland, where they killed. Tens of thousands of people died there, some killed outright and others dying from malnutrition and disease.

Terezín's main hospital was located in a large barrack which was built in 1780 to service military and civilian populations and during World War II became a Jewish hospital. Terezin was a pharmacy, too. It was located in building Q412. In the archives of the Jewish Museum there are several documents related to this pharmacy. They are: daily report on labour deployment in Terezín 1943–1945 (the back of the first document contains the numbers of prominent Jews in the various sectors), graph showing the expenses of the pharmacy in L 305 in Terezín for February 1944, graph showing the expenses of the pharmacy in L 305 in Terezín for February 1944, inventory of the Central Medical Supplies Warehouse in Terezín and notifications of receipt of goods 1942–1945 and notifications of receipt of goods from the central medical supply warehouse to the Economics Department in Terezín 1944.

Most important of them is an inventory of the Central Medical Supplies Warehouse in Terezín and notifications of receipt of goods 1942–1945. This list of medicaments is an important source of knowledge about equipment pharmacies in Terezín. A number of Jewish apothecaries from the Czech lands worked in Terezín pharmacy, but also from abroad.

From the Czech Jewish pharmacists I can mention PhMr. Josef Freund, PhMr. Karel Kürschner, PhMr. Marie Sandová, pharmacy student Ilsa Steinbergová, PhMr. Leo Duschak, student pharmacy Hanna Pachnerová and numerous other. From Germany, for example, it was PhMr. Fritz Silten, owner of pharmacy Kaiser Fridrich Apotheke in Berlin. PhMr. Karel Kürschner was a manager of pharmacy from 1941 to 1944.

In 1944 (23th June) International Committee of the Red Cross visited Terezín. During his inspection, they check the pharmacy and surprised them equipment of pharmacy. Pharmacy, as well as the whole city was just a fake backdrop Nazi prepared for the visit of the Commission. The truth was completely different.

The study was supported by SVV 260 187.

ANTIBIOTIC USE PRACTICES BY PHARMACY STAFF: A CROSS-SECTIONAL STUDY IN SAINT-PETERSBURG, RUSSIAN FEDERATION

BELKINA, T.,[1] DUVANOVÁ, N.,[1,2] KOROLKOVA, A. [3] DUINTJER TEBBENS, J.,[4,5] VLČEK, J.[1]

[1] Department of Social and Clinical Pharmacy, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[2] Pharmacy Lekmin Spořilov, The Polyclinic Spořilov, Prague, Czech Republic
[3] Pharmacy Rodnik Zdorovja and LekOptTorg, Saint-Petersburg, Russian Federation
[4] Department of Biophysics and Physical Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[5] Institute of Computer Science Academy of Sciences of the Czech Republic, Prague, Czech Republic
e-mail: belkinat@faf.cuni.cz

The global emergence and spread of antimicrobial resistance remain a major infection control challenge and a predominant reason for therapy failure. Non-prescription access to antimicrobials is common and self-prescribing is increasingly popular in Russian society. The aim of this study was to assess attitudes of community pharmacists related to antibiotics use and self-medication.

We conducted a cross-sectional study of community pharmacists in Saint-Petersburg and Leningrad region, Russia (n = 410). Personal interviews were conducted using a self-administered questionnaire. The data were analysed using logistic regression and Pearson chi-squared tests.

Of the total of 316 pharmacists (77.07%) who completed the questionnaire, 241 (76.3%) self-medicated with antibiotics. Antibiotics were mostly used to self-treat upper (53.3%) and low (19.3%) respiratory tract infections relying on own knowledge (81.5%), previous treatment experience (49%) and patients' prescriptions (17%). The most commonly used antibiotics were macrolides (33.18%). Characteristics such as age, education and experience were shown to be related to antibiotics use and self-medication.

The study confirms that self-prescribing of antibiotics is a common practice amongst pharmacists in Saint-Petersburg. Pharmacists' personal and professional traits strongly associated with self-medication were identified.

The study was supported by SVV 260 187.

SAFETY OF DIRECT ORAL ANTICOAGULANTS IN PATIENTS WITH CHRONIC RENAL FAILURE

DVOŘÁČKOVÁ, E.,[1,2] STARÁ, D.,[2] PÁVEK, P.,[3] VLČEK, J.[1]

[1] Department of Social and Clinical Pharmacy, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[2] Hospital Pharmacy, Institute for Clinical and Experimental Medicine, Prague, Czech Republic
[3] Department of Pharmacology and Toxicology, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
e-mail: dvore4aa@faf.cuni.cz

Introduction: For many decades, the vitamin K antagonists (VKAs) have been the only oral anticoagulant drugs available for clinical use for the primary and secondary prevention of venous and arterial thromboembolic events. More recently, direct oral anticoagulants (DOACs), namely the direct thrombin inhibitor dabigatran etexilate and the direct factor Xa inhibitor rivaroxaban and apixaban, have been approved for clinical use. Because of their greater specificity, rapid onset of action, and predictable pharmacokinetics, DOACs address several limitations VKAs in day-to-day clinical practice. However, a range of practical questions relating to DOACs including topics such as patient selection, treatment of patients with renal failure.[1]

Objective: The aim of the project was to determine safety of DOACs in patients with renal failure.

Methods: We conducted an observational analysis of 25 patients with chronic kidney disease, eGFR < 60 ml/min.Patients were > 18 years and had stable renal function, and they were > 3 months after renal transplantation, the use of DOACs.

Results: Patients were screened between Januar 1 and December 30, 2015. A total of 25 patients were screened. By three patients had dose reduction during treatment due to a change renal function. Only one patient had dose reduction due to bleeding.

Conclusion: All DOACs are excreted by the kidneys, meaning that in patients with renal failure, the concentration of anticoagulant and its effects increase. Fluctuating kidney function may explain the increased bleeding with DOACs. Monitoring of adverse effects is essential during therapy by patients with renal failure.

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PATIENT ACCESS GAP: THE DELAY BETWEEN MARKETING AUTHORIZATION AND REIMBURSEMENT DECISION ON NEW DRUGS

FUKSA, L.,VOCELKA, M.,VYTŘÍSALOVÁ, M.

Department of Clinical and Social Pharmacy, Faculty of Pharmacy in Hradec Králové,
Charles University, Czech Republic
e-mail: fuksl9aa@faf.cuni.cz

In the Czech and other European healthcare systems, most innovative treatments, including drugs, are being made available to patients only after their inclusion on the list of products or services covered (reimbursed) by the public health care insurance. The national processes on drug pricing and reimbursement are usually strictly regulated, and local legislations must also meet the Transparency Directive 89/105/EEC. In the real world, however, there are still significant challenges to the availability of new and innovative treatments given the complexity and stringency of the drug pricing and reimbursement procedures and also due the attractiveness of the local market itself for the drug manufacturer.

The aim of the present study is thus to evaluate the current availability, i.e. positive reimbursement status, of recently authorized new/innovative drugs in the Czech Republic (CZ), and assess the delay between authorization date and reimbursement decision date.

All centralized procedures with positive decisions on new orginal drugs issued by European Commission between January 2007 and December 2015 were collected. Consequently, the inclusion of the respective drugs on the national CZ reimbursement lists and/or positive reimbursement decision availability were checked, including the timelines.

Of the 348 authorized new drugs issued by European Commission, 164 drugs (47%) have ever been reimbursed in CZ. The average length of the CZ national pricing and reimbursement procedure is 302 (±194) days, while the average overall delay between EU marketing authorization date and CZ reimbursement decision is 585 (±294) days.

The length of the pricing and reimbursement procedure apparently exceeds the limits set by the national legislation and Transparency Directive 89/105/EEC. The next step will be benchmarking CZ and other EU countries concerning the time delay between marketing authorization and reimbursement granting.

THE EFFECT OF NUTRITIONAL SUPPORT ON THE BODY FLUID VOLUMES AND ON CLINICALLY SIGNIFICANT ANTHROPOMETRIC PARAMETERS IN CRITICALLY ILL PATIENTS

JOSKOVÁ, V.,[1,2] PÁTKOVÁ, A.,[1,2] HAVEL, E.,[3] KOVAŘÍK, M.,[1,2] PAVLÍČKOVÁ-AIMOVÁ, P.,[1] ZADÁK, Z.,[2] HRONEK, M.[1,2]

[1] Department of Biological and Medical Sciences, Faculty of Pharmacy in Hradec Králové,
Charles University, Czech Republic
[2] Department of Research and Development, University Hospital, Hradec Králové, Czech Republic
[3] Department of Surgery, University Hospital, Hradec Králové, Czech Republic
e-mail: joskovv@faf.cuni.cz

Many hormonal and metabolic changes are developed in critically ill patients and result in disruption of fluid balance, which is a risk factor for morbidity and mortality in these

patients. Therefore evaluation of fluid balance is one of the basic approaches of correct care in these patients. However the effect of nutritional support on the fluid balance is not specifically known. Another risk factor for prolongation of the period of ICU stay and higher mortality is the loss of muscle mass. The aim of this study was to evaluate the influence of administered nutritional support on those selected parameters in critically ill patients. The study included 14 patients in which was performed 32 examinations using indirect calorimetry and bioelectrical impedance spectroscopy. The average age of the patients was 45.29 ± 18.34 years. Using correlation analysis there has been demonstrated the effect of nutrition on the total fluid output and urine volume. Nutritional intake of energy in kcal d^{-1} ($p = 0.0045$; $r = 0.4890$), carbohydrate intake in g d^{-1} ($p = 0.0099$; $r = 0.4490$) and intake of lipid in g d^{-1} ($p = 0.0208$; $r = 0.4200$) significantly increased production of urine in ml d^{-1}. Only intake of protein ($p = 0.0010$; $r = 0{,}5540$) and carbohydrate ($p = 0.0070$; $r = 0.4676$) decreased the muscle degradation. Application of this knowledge can increase the effectiveness of parenteral and enteral nutrition and contribute to decrease the risk of morbidity and mortality in patients. The effects of the composition of the nutritional support will be validated in following validation study.

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THE EFFECT OF ENERGY AND NUTRITIONAL SUBSTRATES INTAKE ON THEIR OXIDATION AND THE ENERGY EXPENDITURE IN POLYTRAUMA PATIENTS

PÁTKOVÁ, A.,[1,2], JOSKOVÁ, V.,[1,2] HAVEL, E.,[3] KOVAŘÍK, M.,[1,2] PAVLÍČKOVÁ- AIMOVÁ, P.,[1] ZADÁK, Z.,[2] HRONEK, M.[1,2]

[1] Department of Biological and Medical Sciences, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[2] Department of Research and Development, University Hospital, Hradec Králové, Czech Republic
[3] Department of Surgery, University Hospital, Hradec Králové, Czech Republic
e-mail: patkova2@faf.cuni.cz

The optimal amount of supplied energy and nutritional substrates is currently the subject of many professional debates and it is not well known. It is because the metabolism of these patients is completely different from healthy people and varies significantly throughout the critical state. The aim of this project was to find out how the intake of energy and nutritional substrates may affect energy expenditure (EE) and substrate oxidation (SO) in these patients.

The study was performed on 14 polytrauma patients (11 men and 3 women) with 32 examinations. Their mean age was 45.29 ± 18.34 years. Results were obtained after at least 4 hours nutritional support asdministration. REE and SO were measured by indirect calorimetry.

The intake of energy in kcal kg^{-1} d^{-1} ($p = 0.0084$; $r = 0.4582$), carbohydrates in g kg^{-1} d^{-1} ($p = 0.0185$; $r = 0.4141$) and lipids in g kg^{-1} d^{-1} ($p = 0.0208$; $r = 0.4200$) sig-

nificantly influenced EE in kcal kg^{-1} d^{-1}. Only the lipids intake in g kg^{-1} d^{-1} increased the oxidation of lipids in g kg^{-1} d^{-1} ($p = 0.0041$; $r = 0.5085$).

It was demonstrated that the amount of administered nutritional substrates was not proportional to the organism needs. This knowledge can be applied in clinical practice for optimization of the nutritional support composition and thus help to reduce the risk of morbidity and mortality in critically ill patients.

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SAFETY ASSESSMENT OF FOOD ADDITIVES IN TOP-SELLING DIETARY SUPPLEMENTS IN THE CZECH REPUBLIC

STRÁŽNICKÁ, J.,[1] POKLADNÍKOVÁ, J.,[1] JAHODÁŘ, 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

Food additives find use not only in the food industry but also in the pharmaceutical sector. They are present mainly in medicines and in dietary supplements (DS). Adverse reactions of food additives are in the general population 0.01–0.23%, higher in atopic individuals (2–7%).[1]

The aim of this study is to evaluate the frequency of potentially harmful excipients in the best selling dietary supplements in the Czech Republic and to consider their adverse effects. In total, 418 DS were identified, including 54.8% DS for children under 12 years of age. Of these, 66.7% contained at least one additive known to have a negative health effect. On average, there were five additives per DS. The most frequently reported additives were glycerol (28.7%) and titanium dioxide (26.3%). The most commonly found potential adverse effects caused by additives as reported in the literature were gastrointestinal symptoms (50.96%), hypersensitivity reactions (31.34%), or attention-deficit/hyperactivity disorder (6.5%). Food additives should be safe if the acceptable daily intake is complied with; however, some individuals can experience immediate effects (headaches etc.) or long-term effects.[2]

The study was supported by SVV 260 187.

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MEDICATION ADHERENCE TO SEVERE CHRONIC OBSTRUCTIVE PULMONARY DISEASE (COPD)

TOUŠKOVÁ,T.,[1] VYTŘÍSALOVÁ, M.,[1] KOBLÍŽEK,V.,[2] NOVOTNÁ, B.[2]

[1] Department of Social and Clinical Pharmacy, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[2] Pulmonary Department, Faculty of Medicine in Hradec Králové and University Hospital Hradec Králové, Charles University, Czech Republic
e-mail: touskovt@faf.cuni.cz

Inhalation drugs are the basics of pharmacological treatment of COPD. Sufficient adherence to the treatment, including proper application technique and choice of a suitable inhalation system are key factors in the effectiveness of the treatment. The major problem is failure to adhere to application technique. The objectives of the study are to assess adherence to inhaled medication with emphasis on application technique (inhalation adherence) and to study correlates of the adherence among patients with severe COPD in clinical practice.

An observational multicentre study with the participation of 12 centres in the Czech Republic was conducted in cooperation with the Czech Multicentre Research Database of COPD. Eligibility criteria were as follows: diagnosis of COPD and post-bronchodilator FEV1 $\leq$ 60%. The assessment was structured into five steps to be followed while using an inhaler. Adherence to each step was assessed in a dichotomous manner.

A total of 234 patients were available for the analysis of adherence. The assessment of the adherence to application technique revealed that less than 32% of the cohort adhered properly to each of the five steps. For most types of inhalation systems, the highest rate of failure was observed in step 3 (failure to breathe out completely in one breath before taking the medicine with the next breath). Errors in application technique were associated with depression, exacerbations and inspiratory fraction (IC/TLC).

Only one third of patients with COPD was fully adherent to inhalation therapy. The most common error was incomplete breathing out before taking the medicine.

The study was supported by project SVV 260 187. The COPD project is registered in ClinicalTrials.gov with the identifier NCT01923051.

NEGATIVE EFFECTS OF ANTIEPILEPTIC DRUGS ON BONE HEALTH

ZIMČÍKOVÁ, E.,[1] ŠIMKO, J.,[2] KREMLÁČEK, J.,[3] TALÁBOVÁ, M.,[2] FEKETE, S.[4]

[1] Department of Social and Clinical Pharmacy, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[2] Department of Neurology, Faculty of Medicine in Hradec Králové, Charles University and University Hospital Hradec Králové, Czech Republic
[3] Department of Pathophysiology, Faculty of Medicine in Hradec Králové, Charles University and University Hospital Hradec Králové, Czech Republic
[4] Institute of Clinical Biochemistry and Diagnostics, Faculty of Medicine in Hradec Králové, Charles University and University Hospital Hradec Králové, Czech Republic
e-mail: eva.zimcikova@faf.cuni.cz

Negative effects of antiepileptic drugs (AEDs) on bone health are one of the most significant and discussed side effects. More than half of the adult patients with epilepsy treated with "older" antiepileptic drugs have abnormal values of bone mineral density (BMD). Osteopenia was found in many of these cases, osteoporosis was diagnosed to lesser extent. The effect of new generation of AEDs is unknown. Especially in children with epilepsy there is a lack of reliable data on the effects of AEDs on bone. When compared to older AEDs newer AEDs in general have better safety profile with comparable efficacy. However some evidence exists on association between AED medication and decreased BMD in children. Since AED medication usually means long time treatment it is necessary to bring clear information on the effects of AEDs on bone.

The aim of this study was to measure BMD in children treated with AED monotherapy, compare the effects of three commonly used AEDs on BMD and consider some life style aspects with possible influence on bone health. Children between 3–18 years who were receiving either topiramate (TPM) or lamotrigine (LTG) or valproic acid (VPA) for at least 12 months and who were not treated with any other AEDs in the past were enrolled in the study. BMD was examined by dual roentgen absorptiometry (DEXA). Daily calcium intake, physical activity and time of staying outside were assessed by questionnaire and interview.

In the group of 28 children (mean age 179 months, mean treatment duration 38 months) 10; 10 and 8 children were treated with VPA; LTG and TPM, respectively. Osteopenia was found in 8 (28.6%) children – 4 with VPA, 2 TPM and 2 treated with LTG. Z score indicating osteoporosis was not found in any of them. BMD was significantly correlated only with age and BMI (Spearman coef. 0.78, resp. 0.49, $p < 0.05$). Decreased z score was found in children with low physical activity.

According to the cross-sectional design of the study and low number of participants we cannot estimate the risk of particular AEDs on decreased BMD. Further investigation is necessary to find out the impact of AEDs on bone health. However we suggest that regular screening of BMD in children treated with AED therapy should be recommended.