EFFECT OF TRANSIENT HYPOXIA ON LONG-TERM POTENTIATION IN RAT HIPPOCAMPAL SLICES

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Long-term changes in synaptic transmission (ST) as long-term potentiation (LTP) are thought to be substrates for learning and memory. The strength of synapses is not fixed but can be modified by activity, indicating that the brain is plastic. Synaptic plasticity is crucial to the development of the nervous system and thereafter to the ability of an individual to learn and remember new information. Synaptic plasticity is also believed to be important in neurological disorders, such as epilepsy and neurodegeneration, and in recovery from neuronal injury. The aim of this study was to initiate LTP of ST in rat hippocampal slices and to measure the effect of transient hypoxia with hypoglycemia (HYP; 2.5–4.5 min) on LTP. Neurons were stimulated via Schäffer collaterals in the CA3 region using bipolar stainless wire electrode and field excitatory postsynaptic potentials (fEPSP) were recorded from the CA1 region. Recordings were amplified, visualized on the oscilloscope Tektronix 2230, digitalized by the Digidata 1322A with sampling rate of 10 kHz and stored on personal computer for off-line analysis by the AxoScope 9.2 software. The stimulus intensity was reduced to 50% of the threshold value for a population spike generation at the beginning of recording. The stimulus frequency was 0.05 Hz. LTP was induced by the same protocol in all experiments, i.e. by one single train of 100 Hz frequency, 1s duration (High Frequency Stimulation, HFS). The mean value of the fEPSP amplitude monitored during the last ten minutes before application of a single train represented 100%. HFS induced significantly increased amplitude of fEPSP compared to baseline before HFS, measured at the next 40–60 min after a single train. Transient HYP, which initiates short-term failure of ST, was applied 40 min before HFS evoked attenuation of LTP magnitude. The results suggest that transient ischemia could have deleterious effect on learning and memory. These findings call for studying the effect of antioxidants on the induction of LTP in hippocampal slices exposed to oxidative stress.

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DETERMINATION OF NAPHTHOQUINONES AND PHENOLS IN TISSUES OF PLANT SPECIES WITH RESPECT TO EVALUATION OF ANTIOXIDANT ACTIVITY

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Effects of plant secondary metabolites on plants as well as animals have been investigated for many decades. There have been described and studied groups of plant secondary metabolites with positive influence effects on animals. To this group of substances phenolic plant secondary metabolites can be considered.

In the work we aimed on optimization and utilization of high performance liquid chromatography coupled with UV-VIS and amperometric detector for determination of naphthoquinones (1,4-naphthoquinone, lawsone, juglone and plumbagin) and phenols (gallic acid, salicylic acid, quercetin, rutin and flavone). Separation of target molecules was carried out on chromatographic column C18 with reverse-phase. Acetic acid and methanol was used as mobile phase. The other experimental parameters were further optimized. The optimized parameters were as follows: column and detector temperature 42°C, flow rate of the mobile phase 0.75 ml/min. Under these parameters well developed and symmetric signals for the target molecules were measured. The detection limits (3 S/N) for naphtoquinones and phenols were estimated below 1 μM. In addition we quantified the secondary metabolites in plant tissues. The content of naphthoquinones was determined in *Nepenthes*. The methanolic extracts of *Nepenthes* tissues (12 hours long extraction) were analysed by optimized method and high content of plumbagin with low content of lawsone was determined. Further phenolic compounds were determined in tissues of fruits (hawthorn, bilberry, shadbush, honeysuckle). Via content of secondary metabolites we evaluated antioxidant capacity of the selected fruits. We found that hawthorn had considerable antioxidant effect (relative antioxidant capacity was 77%). Based on the results the antioxidant potential at various plant species can be evaluated. Species with considerable antioxidant effects can be also considered *Lonicera cearulea*, L., *Amelanchier canadensis* L., and *Crataegus Brettschneideri*, (C. K. Schneider) Hybr.

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ANALYSIS OF ACE INHIBITORS IN BIOLOGICAL MATERIAL

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This study presents analysis of a selected angiotensin converting inhibitors (ACEI), specially *captopril, enalapril, ramipril, perindopril* and *trandolaril,* and the determination of *ramipril* in human serum using *enalapril* as an internal standard (IST) by high-performance liquid chromatography-mass spectrometry (HPLC-MS). Mass spectra were obtained using a quadrupole analyzer with a atmospheric pressure chemical ionization (APCI).

In this study, the optimal conditions for separation of ACEI in human serum were found. The optimized param-

eters of separation were composition of mobile phase, flow rate, column temperature during measurements.

Chromatographic separations were carried out on a Gemini C18 (2 mm × 150 mm, 5 µm) with gradient elution. Mobile phase consisting of 0.01 M trifluoracetacid in acetonitril, the flow rate was 0.3 ml/min and a column temperature 35 °C.

TDM OF NEW ANTIEPILEPTIC DRUGS – TOPIRAMATE AND LEVETIRACETAM

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This work deals with the development and full validation of HPLC-MS methods for the determination of new antiepileptic drugs *topiramate* and *levetiracetam* in biological samples. Topiramate and levetiracetam belong to the safest antiepileptic drugs in comparision with elderly used anticonvulsants through its high bioavailability, minimal interactions and easy elimination.

Topiramate has been introduced into clinical practice in 1995 and it has been found as very well tolerated and effective anticonvulsant drug (especially for treatment of pharmacoresistant epillepsy).

However the reasons for TDM of topiramate still exist. Among suspicion of non-compliance the most important reasons are interactions with carbamazepine and phenytoin which may significantly decrease the concentration of topiramate, renal impairment and severe hepatic disease. The therapeutic ranges still have not been generally estimated, the serum concentration over 12 mg/l has been associated with slight toxicity. Topiramate can cause ataxia, dysphasia, somnolence, dizziness and anorexia.

Levetiracetam belongs to the safest anticonvulsant drugs without any known pharmacointeractions, wide therapeutic range and minimum side effects. It is not effective in monotherapy of acute seizures and the documentation of effectiveness in generalized seizures is lacking. The major reason for TDM of levetiracetam is the suspicion of non-compliance.

Herein we report rapid and simple, in-house developed and fully validated HPLC-MS methods of topiramate and levetiracetam determination in serum or plasma which are suitable for routine use in clinical laboratory practice.

CHROMOSOMAL DAMAGE AND POLYMORPHISMS IN DNA REPAIR GENES *HOGG1* SER326CYS AND *XRCC1* ARG399GLN IN CHROMIUM-EXPOSED WORKERS

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Welders have been chronically exposed to hexavalent chromium with potential consequences on chromosomal integrity. Our study is focused on the level of chromosomal aberrations with respect to chromium level in the blood of welders as well as on the tentative modulating role of polymorphisms in DNA repair genes *hOGG1* Ser326Cys and *XRCC1* Arg399Gln on chromosomal damage.

The study was performed on 39 welders that have been exposed to chromium for 10.2±1.67 years, and 31 control individuals. Conventional cytogenetic analysis was employed for detection of CAs. XRCC1 and *hGGO1* polymorphisms were assayed for by Taqman SNP genotyping assay ("Assay-by-Demand") using Real-Time allelic discrimination on AB 7500 equipment. Chromium analysis in the blood was performed using the atomic absorption spectrophotometer.

Higher frequencies of CAs were detected in exposed individuals than in controls (1.96% versus 1.55%, respectively), but this difference was not significant. In the exposed group the chromosomal damage consisted predominantly of chromosomal-type of breaks (CSAs; 1.03%), which were approximately two-fold higher as compared to the controls (0.55%). The frequency of chromatid-type breaks was similar in both exposed and control groups (0.92% vs. 1.00%).

Higher pooled CAs was detected in individuals with homozygous wild type polymorphisms in *hOGG1* Ser326Cys as compared to those with heterozygous and homozygous variant genotype (1.83% and 1.57% respectively). After the stratification of the cohort, within control individuals we observed not quite significantly higher frequency of CA associated with wild-type Ser allele in *hOGG1* Ser326Cys (1.71% and 1.20% respectively; p=0.060).

Significantly higher pooled CAs was detected in individuals with homozygous wild type polymorphisms in *XRCC1* Arg399Gln gene as compared to those with heterozygous and homozygous variant genotype (1.33% 1.80% and 2.14% respectively).

The identification of individuals with increased susceptibility to chromium enables to take preventive measures during working process and may contribute to our understanding the effects of chromium on chromosomal integrity.

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TOXICITY EVALUATION OF $AgNO_3$ USING *VIBRIO FISCHERI* TEST

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Silver nitrate is technically the most important compound of silver. The maximum usage of silver nitrate and thereby the maximum volume of wastes is in the

photographic and electrotechnical industries, electrochemical cladding and health services – dental amalgam and infection prevention, in particular. Silver nitrate is easily soluble in water and it is not surprising therefore that many published studies report marked toxicity of silver for aquatic ecosystems. Silver nitrate cauterizes and destroys organic tissues, as it is able to coagulate the proteins. Bacterial bioluminescence toxicity test (BBT) is the commonly used test for determination of toxicity. This test method provides a rapid, reliable, and convenient means of determining acute toxicity.

The aim of our work was to determine effective concentration of silver nitrate, which has the inhibiting effect for the bacterium at 50% (EC50) and 20% (EC20) level after 15 and 30 minutes, respectively. The highest concentration of silver nitrate was 10 μM, higher concentration lead to coagulation of silver ions (such as AgCl).

The inhibition of the bacterium in the concentration 10 μM was 18.5% after 15 minutes and 32% after 30 minutes. The accrual of the inhibition on the concentration was a logarithmic function of concentration. It resulted in our ability to calculate only the effective concentration EC20 after 30 minutes, which is 7.24 μM.

The established values are very interesting, because we were expecting a higher degree of inhibition in these concentrations (due to high toxicity of silver ions). The fact is we cannot use a higher concentration of silver nitrate, mainly due to the occurrence of NaCl in the nutrient substance. As we cannot calculate EC50, the conclusion is that the silver nitrate is not ecotoxic for *Vibrio fischeri*.

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BIOCHEMICAL MARKERS FOR ASSESSING CONTAMINATION OF AQUATIC ENVIRONMENT

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The aquatic environment has become an easily accessible disposal site for xenobiotics and pollutants. The need for assessment of aquatic ecosystem contamination and of its impact on water dwelling organisms has developed in response to rising aquatic environmental pollution, by chemical and industrial contaminants, in the past several decades.

The effect of contamination on fish populations was assessed using biochemical markers and chemical analyses of fish muscle and bottom sediment. Biochemical markers, specifically liver enzymes of the first and the second phase of xenobiotic transformation, namely cytochrome P450, ethoxyresorufin-*O*-deethylase, glutathione-S-transferase and tripeptide reduced glutathione, and biomarker of xenoestrogenic pollution, vitellogenin, were used to assess contamination of the aquatic environment at different river sites in the Czech Republic. The indicator species selected were the male chub (*Leuciscus cephalus L.*) and male brown trout (*Salmo trutta fario*).

Chemical analysis included assessment of the most important inductors of mentioned biochemical markers. The major inductors monitored biomarkers are industrial contaminants which belong to a large group of organic pollutants, (PCB, PAH, PCDD/F, DDT, HCH, HCB and OCS), persistent in the environment

Three different groups of river sites were assessed: the River Tichá Orlice and its tributary Kralický brook, important tributaries of the River Elbe (the rivers Orlice, Chrudimka, Cidlina, Jizera, Vltava, Ohře and Bílina) and major rivers in the Czech Republic (the rivers Lužnice, Otava, Sázava, Berounka, Vltava, Labe, Ohře, Svratka, Dyje, Morava and Odra).

Analyses of biochemical markers and chemical analyses revealed that the most contaminated rivers were the Vltava (site Zelčín), the Labe (site Obříství), and the Ohře (site Terezín). In most cases, elevated biomarker values were found together with elevated levels of the pollutants monitored.

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DARK SIDE OF CHEMOPREVENTIVE COMPOUNDS

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Cancer is the one of leading causes of death in human population. Many naturally occurring agents suggest chemopreventive potential in animal models and epidemiological studies to decrease the incidence of cancers. Beneficial activities of these compounds are usually connected with their anti-proliferation and apoptotic impacts on cancer cells. On the molecular basis, chemopreventive compounds possess health-promoting activity via affecting a carcinogen activation process by inhibiting the formation of ultimate forms of carcinogens and/or stimulating their detoxification.

In order to approve dietary compound to be a human chemopreventive agent the mechanism of its action towards the desired target as well as all other interactions within the body should be known in details. However, although the dietary flavonoids are considered to be chemopreventive antioxidants, they may act as pro-oxidants in systems containing metal such as iron and oxygen. The formation of reactive oxygen species and phenoxyl radicals can damage DNA, lipids, and other biological molecules. Chemopreventive compounds are xenobiotics involved in biotransformation pathways which may interfere with the metabolism of endogenous

compounds. Despite the widespread use of chemopreventive compounds, documentation of their interactions with drugs are sparse. Thus, drug-chemopreventive compound interactions possible result in overdose or lack of therapeutic effect of common drugs. Moreover, phytoestrogens (e.g. isoflavone genistein and coumestrol) may act as endocrine disrupters. In addition, the possible adverse effects arise from the induction of biotransformation enzymes. Flavonoids, e.g. quercetin, galangin, which are ligands of the aryl hydrocarbon receptor, elevate activities of the cytochromes P450 that are responsible for the activation of carcinogens such as meat-derived heterocyclic aromatic amines, benzo[a] pyrene, and aflatoxin B1. While flavonoids effectively inhibit activation of particular carcinogen when present simultaneously, at the same time they initiate a delayed and long lasting induction of cytochromes P450 activating the other carcinogen ingested in food afterwards.

Hence, detailed study on flavonoid interactions from the view point of the modulation and induction of cytochromes P450 activity would be helpful in preventing the activation of food carcinogens and explaining changes in drug metabolism.

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INHIBITION OF THIOREDOXIN REDUCTASE AND GLUTATHIONE PEROXIDASE BY MYRICETIN, QUERCETIN AND RESVERATROL

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Thioredoxin reductase (TrxR) is a selenoprotein that catalyses the reduction of oxidized thioredoxin, using nicotinamide adenine dinucleotide phosphate (NADPH) as the electron donor. The thioredoxin system (i.e. thioredoxin reduktase, thioredoxin and NADPH) together with the glutathione system are regarded as main regulators of the intracellular redox environment. Glutathione peroxidase (GPx) is a selenoprotein that reduces the level of various peroxides occuring in cells using glutathione as an electron donor.

Myricetin and quercetin are flavonols that are different only in one –OH group, resveratrol is a stilben. All these 3 chemicals exert antioxidant activity.

In the present study, the effect of myricetin, quercetin and resveratrol on mammalian TrxR and GPx has been investigated in experiments *in vitro* and *in vivo*.

In *in vitro* experiments, myricetin exhibited the strongest inhibition of TrxR and GPx activity, followed by quercetin. Resveratrol exerted the lowest inhibition.

In *in vivo* experiments, myricetin, quercetin and resveratrol were administered to male ICR mice at the dose of 20 mg/kg b.w. per os once daily for 3 days. Twenty-four hours after the last dose of antioxidants, the activity of TrxR, GPx , the level of lipid peroxidation (LP) and reduced glutathione (GSH) were estimated in liver homogenates. The activity of TrxR was inhibited by myricetin (to 64%, $p<0.05$) and quercetin (to 43%, $p<0.01$) and the activity of GPx was inhibited by myricetin (to 46%, $p<0.01$) and by quercetin (to 64%, $p<0.01$). Resveratrol did not affected the activity of both enzymes. Resveratrol, myricetin and quercetin exerted no influence on lipid peroxidation and GSH level in not exposed animals (male ICR mice).

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THE USE OF HEPATOCYTES FOR THE *IN VITRO* EVALUATION OF CYTOTOXIC AND GENOTOXIC EFFECTS OF OXIDATIVE STRESS: PROTECTIVE PROPERTIES OF CARBOXYMETHYL-CHITIN GLUCAN

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The liver is the main organ for the metabolism of foreign compounds. For this reason hepatocytes represent a suitable system for the evaluation of different influences (detoxifying, antimutagenic and antioxidant), which potentially alter the response to mutagenic/promutagenic compounds. Utilizing primary hepatocyte cultures we addressed two research questions concerning the potential antimutagenic action of carboxymethyl-chitin glucan (CM-CG) with respect to oxidative stress: 1. is the antimutagenic action of this natural compound connected with an alteration of the composition or activities of the biotransformation and detoxification enzymes; and 2. is there a direct interaction of the mutagen with CM-CG? These different models of action can be studied by different treatment protocols used in our experiments: a) pre-treatment with the potential antimutagen; or b) simultaneous treatment of the potential antimutagen with the mutagen.

Oxidative stress was induced by the model free-radical-generating compound 2,3-dimethoxy-1,4-naphthoquinone (DMNQ) or hydrogen peroxide (H_2O_2).

As a measure of genotoxicity, the percentages of DNA in tails of comets by single cell gel electrophoresis were evaluated. The cytotoxicological endpoints analysed were the cell density (number of cells/cm^2), and the percentages of apoptotic and necrotic cells.

DMNQ and H_2O_2, causing DNA single-strand breaks via the formation of $^{\cdot}OH$ radicals, have been demostrated to induce both genotoxic and cytotoxic effects in primary rat hepatocytes resulting in increased percentages of DNA in tails of comets, and apoptotic and necrotic cells accompanied by a decreased cell density. Further investigations were therefore focussed on possible modifications of these parameters by CM-CG.

The results obtained clearly demonstrate that CM-CG (applied before and during treatment) protects primary rat hepatocytes against the genotoxic effects of oxidative stress (DMNQ or H_2O_2), whereas CM-CG itself has no effect on the endpoints of genotoxicity and cytotoxicity studied.

Based on these results we can assume a dual mode of protective action of CM-CG: an alteration of the composition and activities of the biotransformation enzymes towards detoxification and a direct interaction eventually via radical scavenging.

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COMPARISON OF AMOSITE TO CERAMIC FIBRE EFFECT AND THEIR COMBINED EXPOSURE TO CIGARETTE SMOKE – INFLAMMATORY BAL PARAMETERS AND HISTOLOGICAL FINDINGS

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Refractory ceramic fibers (RCF) are used as one kind of asbestos substitutes. Because RCF are relatively durable and some RCF are respirable, they may present a potential health hazard by inhalation. The aim of study was: 1) to find and compare the effects of subchronic exposure to amosite (AMO), refractory ceramic fibres (RCF), cigarette smoke (CS) and combined exposure to AMO+CS and RCF+CS by inflammatory parameters of bronchoalveolar lavage (BAL), 2) to find out if smoking amplifies the possible adverse effect of RCF as it is known after combined exposure to asbestos + CS. Four groups of Wistar rats were treated: 1) intratracheally instilled by saline solution (0.4 ml) – control group; 2) intratracheally instilled by 4 mg of AMO or RCF; 3) exposed only to CS (85 mg of total particulate matter/^{3} air) for two hours daily; 4) exposed to AMO+CS or RCF+CS. After 6 months the animals were exsanguinated and BAL was perfomed. Following BAL parameters were examined: BAL cell count; Alveolar macrophages (AM) count, differential cell count (% of AM, polymorphonuclears and lymphocytes),% of immature AM, binucleated cells, viability and phagocytic activity of AM and histology of lung tissue. The results of our work suggest: serious inflammatory changes in lung after subchronic exposure to AMO, RCF or CF and amplification AMO and RCF effect by CF. 1) There are no great differences between AMO and RCF exposure and their combined effects with cigarette smoke under our experimental conditions in BAL parameters; 2) There are differences of lung tissue injury (fibrosis) between AMO (8) and RCF (5) exposure and no fibrosis effect (0) after cigarette smoke exposure according to histological findings (Wagner scale).

Histology (groups)	C	CS	AMO	AMO + CS	RCF	RCF +CS
cellular change	0	0	3	3	3	3
fibrosis	0	0	8	8	5	5

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ACCESS ROUTES TO BURIED ACTIVE SITES OF CYTOCHROMES P450

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The role of cytochromes P450 in metabolic activation of foreign compounds explains the extensive research interest in these enzymes. Among the interesting topics the P450 studies have to solve is also the intriguing question how the substrate enters the active site (rather closed in some P450 forms), and how the molecules of product are leaving the molecule.

Despite the fact that the number of P450 forms with known X-ray structure vastly increased during the last decade (including several mammalian microsomal forms), and this knowledge contributed very substantially to our understanding of P450 function and mechanism, these data are not completely able to solve the above-mentioned question. As the crystallized molecules were engineered and devoid of membrane anchoring segments, they cannot explain the interaction of P450s with the membrane (often suggested to play important role in substrate entry in the enzyme). This opens the space for other experimental methods (spectroscopic, chemical modification), which may shed additional light onto this problem.

Rabbit CYP2B4 serves as a prototype microsomal CYP of family 2, metabolizing mainly bulky hydrophobic xenobiotics. This isoform has been used as an object of numerous metabolic and structural studies. Recently crystals of N-terminal modified CYP2B4 chimeras, both of the substrate-free form and complexes with ligand inhibitors were prepared, and 3D structure models developed. This P450 form has an active site deeply buried in the protein macromolecule.

In this study, we combined the photoaffinity labeling of the CYP 2B4 with a photolabile substrate analogue, 3-azidiamantane, and molecular modeling (substrate docking). Four diamantane-labeled tryptic fragments were identified by mass spectrometry and sequencing: peptide I (Leu^{359}–Lys^{373}), peptide II (Leu^{30}–Arg^{48}), peptide III (Phe^{127}–Arg^{140}), and peptide IV (Arg^{434}–Arg^{443}). Their positions were projected into CYP2B4 model structures and compared with substrate binding sites, proposed by docking of diamantane. The probable entrance to the proximal heme face (active site) is marked with the labeled Arg^{133}, the remaining label-binding sites may represent alternative entry routes, or play some role

in regulation of enzyme activity or interaction with its redox partners.

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REDUCED PRODUCTION OF REACTIVE OXYGEN SPECIES IN NEUTROPHILS TREATED WITH DIFERULOYLMETHANE

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Diferuloylmethane (curcumin) has been shown to act beneficially in arthritis, particularly through down-regulated expression of proinflammatory cytokines and collagenase as well as through modulated activities of T lymphocytes and macrophages. In this study its effects on activated neutrophils were investigated *in vitro* and in experimental arthritis.

Formation of reactive oxygen species in neutrophils was recorded on the basis of luminol- or isoluminol enhanced chemiluminescence, using a microtitre plate computer-driven luminometer. Adjuvant arthritis was induced in Lewis rats by an intradermal injection of heat-killed *Mycobacterium butyricum*. Diferuloylmethane (50 mg/kg) was administered daily p.o. over a period of 28 days after arthritis induction.

Under *in vitro* conditions, diferuloylmethane (1–100 μmol/l) reduced dose-dependently neutrophil chemiluminescence measured in whole human blood. It counteracted stimulation of neutrophils by receptor-mediated mechanism (opsonised zymosan), direct activation of protein kinase C (PMA, phorbol myristate acetate), as well as by increased calcium concentration (Ca^{2+}-ionophore A23187). A more detailed analysis performed on isolated human neutrophils showed that diferuloylmethane decreased the amount of oxidants both in extra- and intracellular space in concentrations of 0.1 and 10 μmol/l, respectively. Adjuvant arthritis was accompanied by an increased number of neutrophils in blood and by a more pronounced spontaneous as well as PMA stimulated chemiluminescence. Whereas the arthritis-related alterations in neutrophil count and in spontaneous chemiluminescence were not modified by diferuloylmethane, the increased reactivity of neutrophils to PMA was less evident in diferuloylmethane-treated animals.

Diferuloylmethane was found to be a potent inhibitor of neutrophil functions both *in vitro* and in experimental arthritis. As neutrophils are considered to be cells with the greatest capacity to inflict damage within diseased joints, the observed effects could represent a further mechanism involved in the antirheumatic activity of diferuloylmethane.

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CONTRIBUTION OF PHASE II ENZYMES TO METABOLISM OF SILYBIN

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Silybin, the main component of silymarin (flavonoid complex obtained from the seeds of *Silybum marianum*), has been used to date mostly as hepatoprotectant. Recently, silybin has attracted the attention also due to other interesting activities, e.g. anticancer or hypocholesterolemic effects and UV-protective activity. It is known, that silybin can inhibit some activities of CYP enzymes and that is metabolized by CYP2C8 *in vitro* [1]. Contribution of phase II enzymes to the metabolism of silybin, mainly of the UDP-glucuronosyltransferases and sulphotransferases, is presented here.

In this study, at first the human hepatic enzymes of the microsomal fraction were used to prepare individual metabolites of silybin. The HPLC with UV-detection indicated the formation of three glucuronides of silybin; A-7-O-β-D glucuronide, A-20-O-β-D glucuronide and B-20-O-β-D glucuronide. In parallel, human hepatocytes were used to follow this reaction and the respective metabolites were analyzed. In this case, A-20-O-β-D glucuronide and B-20-O-β-D glucuronide of silybin were identified. The highest amount of sulphates of silybin was found in the medium.

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OXIME HI-6 – ANTIDOTE USED IN CASE OF NERVE AGENT POISONINGS

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Oxime HI-6 (1-(2-(hydroxyiminomethyl)pyridinium)-3-(4-carbamoylpyridinium)-2-oxapropane) belongs to the most promising acetylcholinesterase reactivators – antidotes used against nerve agents (sarin, cyclosarin, tabun, VX, etc.). According to the present knowledge, its reactivation potency is the highest compared to other commercial oximes (pralidoxime, obidoxime, trimedoxime, MMB-4). Thanks to the advantages, the development of this compound and its further large-scale production were made within last four years.

In this presentation, we would like to summarize our results to show, what we have done in this topic. We will describe synthesis of twelve different HI-6 salts (sulfate,

chloride, acetate, bromide, phosphate, mesylate, tartrate, iodide, malonate, salicylate, maleinate, tosylate), their rapid TLC and HPLC analysis and their solubility testing. Furthermore, chloride (Cl) and dimethanesulfonate (DMS) salts of the HI-6 were tested *in vitro* and *in vivo* to compare their reactivation differences.

Authors would like to thank to the Ministry of Industry and Trade of the Czech Republic for the Project No. FI-IM2/104.

GENOTOXICITY TESTING USING THE MICRONUCLEUS ASSAY IN THE HUMAN EPIDERM™ 3D SKIN MODEL

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Safety assessment of new products for human use requires genotoxicity testing to ensure their non-carcinogenicity. Current *in vitro* assays have low specificity resulting in a high rate of false positives that may be due to the use of transformed cell lines, non-physiological exposures, and the lack of normal metabolism. To help determine the biological relevance of chemicals that are positive in *in vitro* genotoxicity assays, *in vivo* assays are conducted. However, starting in 2009, European Union cosmetics manufacturers will not be able to use *in vivo* genotoxicity tests. To address this problem, more biologically relevant *in vitro* genotoxicity tests are needed. Since skin has the highest exposure to many cosmetic and personal care products, a skin model will better reflect human exposure and metabolism.

EpiDerm forms a 3D tissue that is highly reproducible, contains a skin-like barrier, and possesses biotransformation capabilities including CYP450, GST, and UDP enzymatic activity. The Micronucleus Assay (MNA) utilizing EpiDerm allows topical application of test materials in a similar fashion to actual human contact. Doses are given 24 hours apart in the presence of 3 μg/ml of Cytochalasin-B in the medium. Cells are harvested from the tissue 24 hours after the last dose. This protocol results in a reproducible population of binucleated cells (49.5% ± 9.6) with a low background% of micronucleated cells (0.1% ± 0.11). We have shown dose-related, statistically significant increases in micronuclei for 3 model genotoxins and for 7 additional rodent skin genotoxins. Also, 4 non-genotoxins were tested at concentrations resulting in <50% cell survival and shown to be negative. Finally, 6 genotoxins that require metabolic activation were positive in the MNA. In conclusion, the MNA that utilizes EpiDerm with inherent metabolic activity is very useful for predicting genotoxicity in a structurally complex tissue of human origin.

SKIN IRRITATION TESTING OF CHEMICALS AND COSMETIC PRODUCTS USING THE RECONSTRUCTED HUMAN SKIN MODEL EPIDERM™

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In vitro toxicology methods are being validated and adopted by regulatory agencies for use as full or partial alternatives to animal experiments. To date, the best results for topical toxicity testing have been obtained with reconstructed human tissues. Particularly for the determination of skin effects that occurs after incidental or intentional exposure, reconstructed human skin models are the most predictive tools *in vitro*. Their use is also included in the proposal of new EU regulatory framework for the Registration, Evaluation and Authorization of Chemicals (REACH) and in the 7th Amendment of the European Cosmetic Legislation.

The ability of reconstructed epidermal models to reliably identify irritation potential of cosmetic products has been demonstrated by number of studies in the past. In an independent EU study related to testing of cosmetic products *in vitro* [1], the reconstructed human tissues models provided sensitivity of 92% and specificity of 100%, resulting in overall concordance of 95% compared to human data. Amongst the assays, EpiDerm model provided the best correlation to human responses (r=0.96). The skin irritation assays developed for reconstructed human skin models EPISKIN and EpiDerm [2,3] were recently recognized by ECVAM as reliable methods for *in vitro* prediction of irritation of neat industrial chemicals [4]. A modified version of the validated EpiDerm protocol was developed by MatTek, which provided increased sensitivity (84%) and thus, now enables more accurate prediction of the irritation effects seen *in vivo* (in the rabbit test). The protocol was recently evaluated in a multi-centre study by 4 independent laboratories to assess its reliability and reproducibility. Based on the published as well as recently produced data, it seems that the skin models have the potential to completely replace skin irritation tests *in vivo*.

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OXIDATIVE DAMAGE CAUSED BY ENVIRONMENTAL CARCINOGENS – EXPERIMENTAL EVIDENCE

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Environmental carcinogens, to which living organisms most frequently are exposed, comprise heavy metals, such as iron and chromium, chemicals used in industry, such as potassium bromate, ionizing radiation, bacterial factors, or drugs, such as 17beta-estradiol. Biological effects of these factors depend on used dose, with carcinogenic effects caused usually by "supraphysiological" doses. The evidence exists for most of known environmental carcinogens that they are able to enhance oxidative stress.

Oxidative stress participates in all the steps of carcinogenesis; at the first step, free radicals damage different molecules – DNA, lipids, and protein, leading directly or indirectly to cancer initiation.

The products of oxidative damage to macromolecules constitute markers of oxidative damage but, simultaneously, they may contribute *per se* to further DNA damage and, consequently, to cancer development. One of the most frequently measured parameter of DNA damage is 8-oxo-2'-deoxyguanosine (8oxodGuo), which is highly mutagenic. Byproducts of lipid peroxidation damage DNA via different mechanisms. Similarly, oxidative damage to proteins may change the properties of enzymes, membranes, etc., contributing to the process of carcinogenesis.

Several antioxidants protect, with different efficacy, against oxidative abuse, exerted by carcinogens. Melatonin (N-acetyl-5-methoxytryptamine) is a well known antioxidant and free radical scavenger. Unambiguous evidence exists that exogenous melatonin is absolutely safe. In the numerous studies, examining several parameters of oxidative damage and using several *in vitro* and *in vivo* models, melatonin has been shown to protect DNA and cellular membranes from the oxidative abuse caused by potential carcinogens.

By reducing the oxidative damage to macromolecules, antioxidants protect against the initiation of cancer. The protection provided by antioxidants against cellular damage, due to environmental carcinogens, make them, especially melatonin, potential therapeutic supplements.

THE EFFECT OF CREATINE CITRATE ON TNE BRAIN ENERGY METABOLIZM DURING SEVERE CHRONIC CEREBRAL HYPOPERFUSION IN THE RAT: *IN VIVO* ^{31}P MR SPECTROSCOPY STUDY

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The bioenergetic dysfunction in the brain plays either a primary or secondary role in the pathophysiology of cell death in neurodegenerative disorders, and even in normal aging. Agents that ameliorate bioenergetic defects may therefore be useful in therapy. Creatine, which increases brain phosphocreatine concentrations, protects against neuronal degeneration in rat models simulating neurodegemerative disorders.

The aim of our study was to evaluate the effect of creatine citrate on brain high-energy metabolites and of the creatine kinase reaction (CK) in the rat brain measured *in vivo* by ^{31}P magnetic resonance spectroscopy comparing with *in vitro* mitochondrial oxidative phosphorylation in aged rats exposed to severe chronic hypoperfusion.

Three vessels occlusion (3VO) was accepted as a reliable model for studying the pathophysiology of vascular dementia, including Alzheimer´s disease (AD) because AD is a vascular disorder with neurodegenerative consequences.

By means of *in vivo* ^{31}P MRS we found that PCr/Pi and also Pi/ATP ratios were significantly changed in rats during 3VO model ($p<0.01$) against control aged rats. These findings reflect of oxidative phosphorylation diminished and global decrease in cerebral metabolic rate. Phosphomonoesters/ATP were significant increased in this group as a consequence of increased phosphorylation. The pretreatment of rats (4 weeks) with creatine in aged rats and also in hypoperfusion group of rats showed the positive effect of creatine only in Pi/ATP ($p<0.001$) and PCr /Pi ($p<0.05$) ratios but not PME/ATP ratio.

The rate constants of CK in the aged rat brains and 3VO model of rats, k_{for} were diminished relative to the brain in intact aged and adult rats perhaps as a consequence of aged related degenerative processes and an inactivation of CK-BB occurs in AD and in other dementias. Our results suggest that high-energy phosphate metabolite levels and dynamic parameter k_{for} could reflect a microvascular degeneration in case of aged and aged-VD rats or/and at the same time the dysfunction of the brain CK system under AD conditions. We suppose that our findings could serve as sensitive *in vivo* MRS indicators of therapeutic efficacy in the case AD and vascular dementia.

This work was facilitated by the support of the Grant AV 4/2006/08 and Technology Agency, Grant APVV-21-022004.

THE EVALUATION OF REACTIVATING, THERAPEUTIC AND NEUROPROTECTIVE EFFICACY OF NEWLY DEVELOPED OXIMES AND CURRENTLY AVAILABLE OXIMES AGAINST TABUN BY *IN VIVO* METHODS

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The potency of four newly developed bispyridinium compounds (K156, K203, K206, K269) in reactivating tabun-inhibited acetylcholinesterase, eliminating tabun-induced lethal toxic effects and reducing tabun-induced neurotoxic signs and symptoms was compared with commonly used oximes (obidoxime, trimedoxime, the oxime HI-6) using *in vivo* methods. Studies determining percentage of reactivation of tabun-inhibited blood and tissue acetylcholinesterase in poisoned rats showed that the reactivating efficacy of newly developed oxime K203 is comparable with obidoxime and trimedoxime in blood and higher than the reactivating potency of trimedoxime and obidoxime in diaphragm and brain. On the other hand, the potency of other studied newly developed oximes to reactivate tabun-inhibited acetylcholinesterase does not prevail the reactivating efficacy of obidoxime and trimedoxime. All newly developed oximes were also found to be relatively efficacious in the elimination of lethal toxic effects in tabun-poisoned mice although only

the oxime K203 is able to decrease the acute toxicity of tabun nearly two times. The therapeutic efficacy of newly developed oximes corresponds to their potency to reactivate tabun-inhibited acetylcholinesterase, especially in diaphragm and brain. On the contrary to obidoxime and trimedoxime, the oxime HI-6 is not effective oxime in reactivation of tabun-inhibited acetycholinesterase and in eliminating lethal effects of tabun. Based on the evaluation of the neuroprotective efficacy of oximes, only one newly developed oxime (K203) was found to be effective for a decrease in tabun-induced neurotoxicity within 24 hours after tabun sublethal poisoning. The neuroprotective efficacy of commonly used oximes (obidoxime and HI-6) as well as another newly synthesized oxime (K206) was significantly lower in comparison with K203 according to the number of eliminated tabun-induced neurotoxic signs at 24 hours after tabun challenge. Thus, among newly developed oximes, only K203 is a suitable reactivator of tabun-inhibited acetylcholinesterase for the replacement of commonly used oximes for the antidotal treatment of acute tabun poisonings.

The study was supported by the grants of Ministry of Defense MO0FVZ0000501 and OPUOFVZ200603.

MICRONUCLEUS FREQUENCY IN LYMPHOCYTES OF AIRCREWS OCCUPATIONALLY EXPOSED TO RADIATION AND STRESS FACTORS

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Airline pilots experience chronic low-dose exposure to high-energy galactic cosmic radiation. Epidemiological studies suggest that there may be a causal relation between cancer incidence in flight personnel and exposure to cosmic radiation. The increasing use of air travel shows the need for biomonitoring studies and risk assessment of flight personnel. Cytogenetic analysis of human lymphocytes is considered to be a valid biomarker for prediction of cancer risk.

We measured chromosomal damage in the stimulated peripheral lymphocytes by micronucleus cytokinesis block assay, a well-validated methodology, which provides a measure of both chromosome breakage and chromosome loss. A total of 98 subjects were recruited from two airlines, 58 pilots (57 men and 1 woman, average age 37 yr), and a reference group of 40 ground crews, similar in age and life style, without a history of frequent airline travel (all men, average age 32 yr). All study participants signed an informed consent form and the Ethical Committee of the Slovak Medical University in Bratislava approved the study. We analysed 2000 binucleated lymphocytes (BN) per person. Scoring criteria for selection of BN cells and MN were set according to the criteria of HUMN project.

We did not find a statistically significant difference in the frequencies of micronuclei between monitored groups. The number of micronuclei in the reference group of ground crews was 1.95 ± 0.28 compared with 1.83 ± 0.24 in the group of pilots.

This project has been supported by the Grant Agency of Ministry of Health of Slovak Republic, MZ 2005/42-SZU-20.

RECONSTRUCTED HUMAN TISSUE MODELS AND THEIR POSITION IN STRATEGY OF REGISTRATION, EVALUATION AND AUTHORIZATION OF CHEMICALS (REACH)

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The new EU Chemical Directive REACH came into force on June 1, 2007 with increased demand on manufacturers and importers to investigate the effects of chemicals on human health. To prevent an increase in the number of animal experiments, REACH allows alternative tests to be used for toxicity testing. Reconstructed human tissue (RHT) models closely mimic native tissues and thus can be used for such purposes. An advantage of the commercial RHT models is that their characteristics can be precisely controlled and evaluated for long-term reproducibility (Rispin et al., Regul. Toxicol. Pharmacol. 45 (2), 97-103, 2006). In addition tests with RHT models for topical toxicity testing are cost-effective and deliver faster and highly reproducible results. RHT-based assays for skin corrosion and irritation testing are already validated and assays for skin corrosion have reached full regulatory acceptance at the OECD level (OECD TG 431). Other *in vitro* RHT methods have completed pre-validation testing (photo-toxicity, eye irritation, genotoxicity) or will soon enter the pre-validation process. Nowadays, RHT models provide cost effective, sophisticated and reliable tools for testing of chemicals within the REACH program.

SEX-RELATED DIFFERENCES IN MYOCARDIAL CONNEXIN-43 EXPRESSION CAN AFFECT LETHAL ARRHYTHMIA SUSCEPTIBILITY

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Clinical and experimental data have identified sex differences in incidence of cardiac diseases and/or lethal arrhythmias. The cellular mechanisms responsible for these disparities are still not fully elucidated. Cell-to-cell communication ensured by gap junction conexin-43 (Cx43) channels plays a crucial role in myocardial tissue homeostasis and synchronisation. Therefore, we hypothesized that expression and/or distribution of Cx43 may differ between males and females.

Aim of this study was to examine both myocardial Cx43 and susceptibility of the heart to life-threatening

arrhythmia, ventricular fibrillation (VF), in non-hypertensive and hypertensive male and female rats.

Experiments were conducted on aged (>1 year-old) male and female normotensive Wistar and spontaneously hypertensive (SHR) rats. Ventricular tissue taken from excised hearts of anesthetized rats was immediately frozen in liquid nitrogen and processed either for in situ immuno-detection or Western-blotting of Cx43 using mouse MAB. Susceptibility to VF was examined in isolated heart preparation according to Langendorff using either electrical burst stimulation or hypokalemic perfusion.

Both male and female SHR rats were more vulnerable to VF compared to Wistar counterparts. In correlation, Western-blotting of Cx43 revealed significantly lower ventricular Cx43 expression in hypertensive than normotensive rats. In addition, all SHR hearts exhibited abnormal myocardial distribution of Cx43, i.e. enhanced amount of lateral, side-to-side Cx43-positive gap junctions. Female rats, both SHR and Wistar were significantly less susceptible to VF comparing to males counterparts. It correlated with significantly higher Cx43 expression in female comparing to male rats.

Taken together these findings indicate that higher level of myocardial Cx43 expression is linked with lower lethal arrhythmia susceptibility and vice versa. This fact can explain, at least in part, sex-related differences in incidence of life-threatening arrhythmias.

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THE USE OF MELATONIN TO COMBAT MUSTARD TOXICITY

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Among the most readily available chemical warfare agents, sulfur mustard (SM) has been the most widely used chemical weapon. The toxicity of SM as an incapacitating agent is of much greater importance than its ability to cause lethality. Oxidative stress is the first and key event in the pathogenesis of SM toxicity. The involvement of inducible nitric oxide (iNOS) in SM toxicity, however, also leads to elevated nitrosative stress; thus, the damage caused by SM is nitro-oxidative stress because of peroxynitrite ($ONOO^-$) production. Once $ONOO^-$ is formed, it activates nuclear factor-κB (NF-κB) and activator protein-1 (AP-1) leading to pro-inflammatory gene expression thereby promoting inflammation; additionally, $ONOO^-$ directly exerts harmful effects by damaging all biomolecules including lipids, proteins and DNA within cells. DNA damage is sensed by an important DNA repair enzyme, poly (ADP-ribose) polymerase (PARP); this enzyme repairs molecular damage by using nicotinamide adenine dinucleotide (NAD^+) as a substrate. Over-activation of PARP, due to severe DNA damage, consumes vast amounts of the respiratory coenzyme NAD^+ leading to a cellular energy crisis. This pathophysiologic mechanism eventually results in cellular dysfunction, apoptosis or necrosis. Therefore, classic antioxidants may have limited beneficial effects on SM toxicity. Melatonin is a multifunctional indolamine which counteracts virtually all pathophysiologic steps and displays significant beneficial effects against $ONOO^-$-induced cellular toxicity. Melatonin has the capability of scavenging both oxygen and nitrogen-based reactants including $ONOO^-$ and blocking transcriptional factors which induce pro-inflammatory cytokines. The delayed toxicity of SM, however, currently has no mechanistic explanation. We propose that epigenetic aberrations may be responsible for delayed detrimental effects of mustard poisoning. Therefore, as a putative epigenetic modulator, melatonin may also be beneficial to subjects with delayed toxicity of SM.

RETINOIC ACID INDUCED NEURONAL DIFFERENTIATION OF P19 EMBRYONAL CARCINOMA CELLS IS MODULATED BY INTRACELLULAR REDOX STATE

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Retinoic acid (RA), the derivative of retinol, plays significant roles in the regulation of cell proliferation, differentiation, and apoptosis. *In vitro*, the pluripotent embryonal carcinoma (EC) and embryonic stem (ES) cells are induced by RA to differentiate into various lineages. It was documented previously that growth in serum-free media itself committed EC cells to neural differentiation and the addition of RA intensified this effect. Signaling pathways and transcriptional factors involved in embryonal cell differentiation are suggested to be sensitive to redox status of intra-cellular environment. Thus, intracellularly produced reactive oxygen species can modulate differentiation of EC/ES cells.

Herein, selected antioxidants (glutathione, N-acetyl cysteine, and ascorbic acid) together with inhibitors of intracellular ROS production (diphenylene iodonium chloride and apocynin) were tested to modulate RA-induced differentiation of P19 cells to neural-like cells. Interestingly, RA significantly increased intracellular production of reactive oxygen species which was inhibited by antioxidants. The RA-induced differentiation of P19 cells into neural-like cells was documented by downregulation of markers of undifferentiated stage of EC cells (E-cadherin and Oct-4) up-regulation of markers of neural cells (N-cadherin and III β-tubulin). To further characterize mechanism of antioxidant modulation of RA-induced EC cell differentiation an activity of RA-directed promotor the retinoic acid responsive element (RARE) was evaluated by luciferase reporter

assay. Antioxidants significantly inhibited RA-induced activation of RARE.

Obtained data suggest a role of reactive oxygen species in RA-induced embryonal pluripotent cell differentiation to neural lineages.

ANALYSIS OF IRON PARTICLES IN THE HUMAN SPLEEN

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Iron can be found in human body mainly in the form of ferritin. Ferritin creates spherical formation with the size of 12 nm with 8 nm ferritin core. Physiological ferritin consists of crystalline ferrihydrite and amorphous iron hydroxide, pathological ferritin consists of wüstite (FeO) and magnetite – like structure.

Sections of three samples of human spleen with diagnosis of hemosiderosis, hereditary spherocytosis and reference spleen were prepared for investigation in light microscopy, transmission electron microscopy, energy-dispersive X-ray microanalysis (EDX) with scanning electron microscopy (SEM), X-ray fluorescence (XRF) and ^{57}Fe Mössbauer spectroscopy.

Iron depositions in samples with diagnosis of hemosiderosis and hereditary spherocytosis were seen after Perls' Prussian Blue stained. Multielemental composition was seen by EDX and XRF analysis. The results from Mössbauer spectroscopy indicate possible phases of the iron oxides present in the studied tissues (Table).

Phase of iron oxide	Isomer shift [mm/s]	Quadrupole splitting [mm/s]
Lepidocrocite gamma-FeO(OH)	0.37	0.53
Feroxyhyte delta-FeO(OH)	0.36	0.69
Ferrihydrite $5\,Fe_2O_3 \cdot 9\,H_20$	0.35	0.71
$FePO_4$	0.38	0.80

Ferrihydrite contains various amount of phosphorus that influences extensively the morphology and iron oxidation [1]. Additional factors influence ferrihydrite phase transformation and biomineralization are chemical composition [2] and pH [3].

Major role in resistance to iron-induced oxidative damage plays antioxidant defenses involving thiol (-SH) metabolism. Cornell et al. [4] found ferrihydrite transformation under the influence of cysteine at pH 6. We suppose that pH and chemical elements are significant factors influence biomineralization of iron in the human spleen.

Mössbauer spectroscopy of studied tissues revealed different phase of iron oxide in the human spleen. Multielemental composition of iron particles was found by EDX and XRF analysis. We suppose that pH and chemical elements are significant factors influence biomineralization of iron in the human spleen.

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CHRONIC LOW-DOSE L-NAME TREATMENT EFFECT ON CARDIOVASCULAR SYSTEM OF RATS WITH VARIOUS FAMILY HISTORY OF HYPERTENSION: DETRIMENT OR BENEFIT?

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NG-Nitro-L-arginine methyl ester (L-NAME) is a non-specific nitric oxide (NO) synthase inhibitor, commonly used for the induction of NO-deficient hypertension. The aim of this study was to investigate the effect of chronic low-dose (1.5 mg/kg/day) administration of L-NAME on NO production, vascular function and structure of the heart and selected arteries of two rat strains with various family history of hypertension. Adult male Wistar and borderline hypertensive rats (BHR, offspring of SHR dams and Wistar sires) were treated with L-NAME in the dose of approximately 1.5 mg/kg/day in drinking water for 8 weeks. Basal blood pressure (BP) of Wistar and BHR (determined by tail-cuff) was 112±3 mm Hg and 136±2 mm Hg. L-NAME significantly elevated BP on weeks 3 and 6 vs. control in both Wistar and BHR. After this period, BP of L-NAME-treated Wistar rats returned to the control values while BP of BHR reamined still significantly elevated vs. control. There were no alterations in the left ventricle-to-body weight ratio, cross-sectional area and wall thickness/diameter ratio of the aorta and the femoral artery of L-NAME treated Wistar rats. By contrast, chronic administration of L-NAME to BHR led to left ventricular hypertrophy and to the increase in the cross-sectional area and wall thickness/diameter ratio of the femoral artery without alterations in the aorta. Additionally, L-NAME inhibited the NO synthase activity in the left ventricle in BHR, while in Wistar rats NO synthase activity in the left ventricle and aorta was significantly elevated. Endothelium-dependent acetylcholine-induced vasorelaxation of the femoral artery in Wistar rats was enhanced after L-NAME administration, but no effect of L-NAME was observed in BHR. The data suggest that chronic low-dose L-NAME treatment can increase NO production and vasorelaxation in normotensive rats without negative structural changes in the heart and vessels. On the other hand, the same low dose can cause the detriment to BP regulation, NO production and structural remodeling of the cardio-

vascular system in rats with positive family history of hypertension.

The study was supported by the VEGA-2/7064/28 and APVT-51-018004.

EXPRESSION OF RECOMBINANT CYTOCHROME b_5 AND ITS UTILIZATION IN STUDIES ON ELLIPTICINE METABOLISM BY CYTOCHROMES P450 1A1 AND 1A2

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Cytochrome b_5, located in membrane of endoplasmic reticulum, is a heme protein with molecular weight of 16 800. Cytochrome b_5 has been shown to influence some cytochrome P450 (CYP)-mediated reactions. Recently, we found that cytochrome b_5 isolated from rabbit liver microsomes and reconstituted with CYP1A1/2 and NADPH:CYP reductase modulates the oxidation of anticancer drug ellipticine. Two major explanations are direct electron transfer from cytochrome b_5 and conformational effects in the absence of electron transfer. For elucidation of the modulation mechanism is neccessary to evaluate not only the effect of native cytochrome b_5, but also the effect of the apo-cytochrome b_5, on ellipticine oxidation. Therefore a heterologous expression of apo-cytochrome b_5 in *Escherichia coli* BL-21 (DE3) Gold cells with a T7 polymerase/promotor system was utilized for preparation of this protein.

The rabbit cytochrome b_5 gene was prepared from synthetic oligonucleotides using PCR, cloned into pUC19 plasmid and amplified in DH5α cells. The final gene sequence was verified by DNA sequencing. The sequence coding cytochrome b_5 was cleaved from pUC19 by NdeI and XhoI restriction endonucleases and re-cloned to the expression vector pET22b. This vector was used to transform *E. coli* cells by heat shock. Culture was grown in Luria Broth medium at 37°C in a Orbital shaker at 200 RPM. Expression was induced with IPTG. Cytochrome b_5, produced predominantly as the apo-form was isolated from purified membranes by ionex chromatography on DEAE-Sepharose. The apo-cytochrome b_5 reconstituted with heme reveals the same oxidized and reduced absorbance spectrum as native cytochrome b_5 and was found to be reduced also with NADPH:CYP reductase. The effect of the purified native cytochrome b_5 and recombinant apo-cytochrome b_5 on oxidation of ellipticine by CYP1A1 and 1A2 reconstituted with NADPH:CYP reductase is studied in details.

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THE EFFECT OF SECONDARY METABOLITES ISOLATED FROM INDOOR MICROMYCETES ON LUNG CELLS – *IN VITRO* STUDY

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The indoor pollution in buildings contaminated with micromycetes has specific components: spores and secondary metabolites from micromycetes. The effect of endo- and exometabolites from micromycetes isolates (*Aspergillus ustus, Aspergillus versicolor, Penicillium chrysogenum, Stachybotrys chartarum*) were studied *in vitro* on isolated lung cells. The toxicological most important types of lung cells were used in this study: alveolar macrophages (AM), alveolar epithelial type II cells (isolated from rats) and non-ciliated bronchiolar Clara cells (isolated from mice). The isolated cells were cultivated with fungal isolates, the cultivation was terminated after 24 h. The cytotoxicity was evaluated in cultivation medium by ToxiLight BioAssay Kit, the activity of marker enzymes was estimated: acid phosphatase in alveolar macrophages, alkaline phosphatase in type II cells. The changes on the cell surface of type II cells were demonstrated by staining with *Maclura pomifera lectin*. The control cells showed positive staining of membranes whilst the staining of cells cultured with metabolites revealed fragmentation of the membranes. The activities of enzymes in cells decreased with enhanced concentration of metabolites. The cytotoxic effect was confirmed in medium. The results showed toxic effect of all tested endo- and exo-metabolites even in very low concentration (0.1 μg/ml).

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TIME COURSE OF REDOX IMBALANCE INJURY IN SARCOPLASMIC RETICULUM VESICLES IN RATS SUFFERING FROM ADJUVANT ARTHRITIS

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Adjuvant arthritis (AA), an animal model of rheumatoid arthritis (RA), was induced by intradermal administration of *Mycobacterium butyricum* (MB) to the base of the tail of Lewis rats. Oxidative stress contributes to the pathogenesis of chronic inflammatory diseases including RA or AA and is tightly associated with imbalance of calcium levels regulated by Ca-pumps including Ca-ATPase from sarcoplasmic reticulum (SERCA) of skeletal muscles.

The content of protein carbonyls was measured by ELISA in control sarcoplasmic reticulum (SR) vesicles,

as well as in vesicles from days 7, 14, 21 and 28 after MB injection. Significant elevation of protein carbonyls was observed only on day 21, the level of protein carbonyls was recovered to control level on day 28. The activity of Ca-ATPase was measured by NADH-coupled enzyme assay. Over the same period, activity of SERCA from skeletal muscles of hind paws was inhibited only on day 21 and it was significantly increased twice on day 28. The modification of Ca-ATPase activity was observed with respect to both substrates, Ca^{2+} as well as ATP. Neither alterations in SH groups nor lipid peroxidation in SR vesicles were observed in the complete time course of AA development. According to electrophoretic studies on SDS-PAGE, no changes in SERCA were indentified over the period from day 0–28 after MB administration.

We conclude that the decrease of calcium regulating enzyme Ca-ATPase on day 21 was in correlation with deamination of posttranslational amino acids. This process was reversible:

after recovering protein carbonyls on day 28, the increase of Ca-ATPase activity suggests development of adaptation mechanisms.

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PERFLUORINATED COMPOUNDS: OCCURENCE AND RISK PROFILE – A REVIEW

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Perfluorinated compounds such as perfluorooctane sulfonate (PFOS) and perfluorooctanoid acid (PFOA) are emerging enviromental pollutants, which are used mainly as surface treatment chemicals, polymerization aids and surfactants, etc. They are ubiqiutous, persistent and bioaccumulative in the enviroment. PFCs (otherwise PFAAs) are being proposed as candidates for a new class of POPs. Although tests in rodents have demonstrated numerous negative effects of PFCs, it is still unclear wheather the exposure to perfluorinated compounds may also damage human health. This review provides an overview of the recent advances in toxicology and toxicokinetics, monitoring data now available for the enviroment, wildlife, and humans and tries to explain the mechanisms of PFCs´actions.

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DETERMINATION OF CDT BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC) IN HUMAN SERUM

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High prevalence of alcohol abuse is an relevant public health problem.

For identification, alcoholism related questionnaires and laboratory tests are used. Currently, the CDT (carbohydrate-deficient transferrin) is most specific biochemical marker for detection of alcohol abuse and for monitoring abstinence during treatment compared to other markers: GMT, AST, MCV. The diagnostic specifity is 80–100% and diagnostic sensitivity is about 60–70%.

Carbohydrate-deficient transferrin is defined as asilo-, monosialo- and disialotransferrin isoforms. Transferrin is synthesized mainly in the liver as a glykoprotein. The two oligosacharide chains have a bi- or triantennary structure and a different amount of bounded sialic acid determines the transferrin isoforms: from asialo- to heptasialotransferrin. tetrasialo transferrin is the most commonly occuring isoforms in human serum. Isoforms with a low rate of sialylation (asialo, monosialo, disialo) are present at very low concentration. The presence of these isoforms is elevated in harmful use of alcohol.

Chronic alcohol abuse (>60g ethanol per day) leads to increasing the value of carbohydrate deficient transferrin.

Analytical methods are available based on isoelectric focussing, HPLC, imunnoassay and capillary zone electrophoresis.

In this work we compare two diferent methods for CDT determination: imunnoassay and high performance liquid chromatography with UV detection.

THE EFFECTS OF DITHIADEN ON NITRIC OXIDE PRODUCTION BY RAW 264.7 CELLS

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As reported in our previous studies, dithiaden (an antagonist of histamine H1-receptor, used clinically as an anti-allergic or anti-emetic drug) in a concentration range of 5×10^{-5}–10^{-4} M decreased the production of reactive oxygen species by phagocytes. In this study, the influence of dithiaden on nitric oxide (NO) production by macrophages stimulated by lipopolysacharide was investigated. Murine RAW 264.7 cells (2.5×10^6/well) were preincubated in the absence/presence of dithiaden for 60min and subsequently stimulated by 0.1μg/ml bacterial lipopolysacharide. After 24-hour incubation in 5% CO_2 at 37°C, a cell supernatant was collected and cells were lysed. NO production was determined as a concentration of nitrites (the end product of NO metabolism) accumulated in the cell supernatant spectrophotometrically via Griess reaction and an expression of inducible nitric oxide synthase (iNOS) in cell-lysates was evaluated using Western blot analysis. Scavenging properties of dithiaden against NO were evaluated amperometrically. Our data demonstrate that dithiaden in the concentration of 5×10^{-5}M decreased the accumulation of nitrites in cell supernatants. It correlated with the inhibition of iNOS protein expression in cells. Amperometrical analyses did not show any scavenging properties of dithiaden against

NO. It suggests that the inhibition effect of dithiaden on macrophage NO production is caused by the suppression of iNOS protein expression.

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EFFECTS OF FLAVONOIDS ON CYTOCHROMES P450

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The increasing incidence of gastrointestinal tract cancer, namely of the intestines and rectum, poses a very serious threat. Dietary composition is one of the factors raising the probability of the carcinoma incidence. Numerous compounds in human diet, e.g. drugs and dietary supplements, are inducers of cytochromes P450 (CYP), the enzymes responsible for xenobiotic metabolism and carcinogen activation. Many natural flavonoids, which are considered to be chemopreventive compounds, may also exert inductive effects on cytochromes P450. Therefore, the aim of this project is to indicate which flavonoids influence the food carcinogen activation caused by cytochromes P450, mainly CYP1A subfamily.

We investigated the effect of 6 flavonoids (β- and α- naphthoflavone, flavone, flavanone, rutin, morin) on CYP1A and CYP2B induction in liver and colon tissues after *p.o.* administration to male Wistar rats.

For the immunochemical identification of the cytochrome P450 protein, we used Western blotting technique. Ethoxyresorufin-*O*-deethylase (EROD), methoxyresorufin-*O*-demethylase (MROD) and pentoxyresorufin-*O*-dependentylase (PROD) activity assays were used as a marker for the activity of CYP1A1, CYP1A2, CYP2B1/2, respectively.

In liver microsomes, we demonstrated the induction of CYP1A1 after administration of three unsubstituted flavonoids (β- and α- naphthoflavone, flavone), and of CYP1A2 moreover after flavanone treatment. These results are in accordance with the elevation of marker activities EROD and MROD. In addition, the strong induction of CYP2B was observed in flavone liver microsomes, which correlates well with the > 7fold increase of PROD activity, compared to control microsomes. However, in colon microsomes, the induction of CYP1A1 was observed after β-naphthoflavone, morin and rutin treatment, which is also supported by the increase of EROD activity.

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METALLOTHIONEINS IN BRAIN TISSUES OF RATS AND THEIR INTERACTIONS WITH METALLOPROTEINASES

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Metallothioneins (MTs) are a group of low molecular weight (about 6.5 kDa) single-chain proteins rich in cysteine. Four major isoforms (MT-1 – MT-4) have been identified in mammals. The specific functional roles of MTs isoforms and their molecular interactions are not clear yet. The neuroprotective function of MT-1 and MT-2 seems to be important. MT-1 overexpression after brain injury stimulates the astroglial responses including the expression of anti-inflammatory cytokines, matrix metalloproteinases (MMP), and many others. Moreover the amounts of the delaying brain tissue damage consisting of oxidative stress, neurodegeneration and apoptotic cell death are much reduced. The aim of this work was to quantify the content of MTs in brain tissues of rats with various zinc administration and to investigate interaction between MMP and MTs.

Male Wistar rats were used in our experiments. The electrochemical measurements were performed with 747 VA Stand instrument connected to 746 VA Trace Analyzer and 695 Autosampler or AUTOLAB Analyzer connected to VA-Stand 663.

Primarily we aimed our attention on determination of MTs level in brain tissues of rats using differential pulse voltammetry Brdicka reaction. The rats were exposed to various doses of zinc(II) ions. We found that zinc(II) doses affected MTs level. This phenomenon could be related with enhancing of activity of zinc-dependent proteins such matrix metalloproteinases. Therefore the second main aim of this work was to propose and optimize the chronopotentiometric stripping analysis in connection with adsorptive transfer technique for MMP-9 detection. The method has been further employed to investigate MMP-9 interaction with collagen and MTs. We optimized the following parameters: time of the accumulation of the sample on HMDE surface and the composition of supporting electrolyte. The suitable parameters were accumulation time of 90 s for both MMP-9 and collagen and supporting electrolyte Britton Robinson buffer (pH 5). It clearly follows from the results obtained that this technique can be considered as promising tool to investigate MMP-collagen or MMP-MTs interactions.

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