MERCURY IN HUMAN HAIR AS AN INDICATOR OF THE CONSUMPTION OF FISH AND QUALITY OF ENVIRONMENT

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Mercury and most of its compounds are extremely toxic and are generally handled with care. They can be inhalated and absorbed through the skin and mucous membranes. The most toxic forms of mercury are its organic compounds such as dimethylmercury and methylmercury. Fish show a natural tendency to cummulate mercury. Methylmercury is produced by microbial methylation in sediments then it infiltrates the food chain and is consequently accumulated in fish. Fish are the main source of methylmercury contamination in people. In the hair, we can monitor long-term exposure of mercury. The content of mercury changes primarily with frequency of fish consumption.

The aim of our study was to compare mercury content in the hair of children with different fish consumption (increased or reduced). Total mercury content in hair was determined by the direct method of cold vapours using the AMA-245 analyzer. Total of 179 hair samples from the children (10–15 years old) were analyzed. In this study were compared following sites: Neratovice (42 pieces), Jeseníky (48 pieces), Praha (59 pieces) and Olsztyn in Poland (30 pieces). Every sample was accompanied by answer sheet including data on age, gender, region and fish consumption. The correlation between fish consumption and mercury content in hair was estimated.

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THE DEVELOPMENT OF NEW REACTIVATORS OF ACETYLCHOLINESTERASE

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Nerve agent poisonings are conventionally treated using a combination of a parasympatolytic drugs (atropine mostly) to counteract the accumulation of acetylcholine at synaptic clefts and AChE reactivators (pralidoxime or obidoxime) to reactivate inhibited AChE.

At our institute, we are interested in searching for new more potent AChE reactivators. For this purpose, we have established a universal process for development of new AChE reactivators. This development process consists of several steps, which are fully connected. They state as follows – description of the nerve agent action on the molecular basis (molecular design), prediction of the biological active structure of AChE reactivators (artificial neural networks), their synthesis, *in vitro* evaluation of their ability (potenciometric titration and Ellman´s method), *in vivo* studies (therapeutic index, LD_{50} of newly synthesized reactivators, reactivation in different tissues, neuroprotective efficacy).

In this presentation, results obtained using this process within last five years will be presented.

The study was supported by the grant of Ministry of Defence, No. FVZMO0000501.

PHARMACOKINETICS AND ORGAN DISTRIBUTION OF FLUORESCEINE AS A DIAGNOSTICS FOR CELLS-CONFOCAL LASER ENDOMICROSCOPY OF GASTROINTESTINAL TRACT (EXPERIMENTAL PIG)

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Confocal laser scanning endomicroscopy (CLSE) seems to be a key technology for obtaining endoluminal non-invasive biopsies. This technique may make histological diagnosis by virtual histology possible [1]. The principle of CLSE: a laser light source delivers blue excitation light at a wavelenght of 488nm. Fluoresceine (FSC) in the tissue absorbs this light and emits green-yellowish light at a longer wavelenght 510–580nm by itself. Only fluorescence light coming from a specific focal plane is detected afterwards by the endomicroscopy system. The aim of experimental pre-clinical study as a preparing phase for clinical application was to specify the diagnostic optimum level of FSC (after its intra-venous adminstration) in the tissue of particular gastrointestinal segments and to delimitate the potential toxicological risks following the relations of its organ distribution. For this purpose, there was designed following experimental scheme in pigs (30–35 kg of body weight): a) determination of the plasma time profile of FSC concentrations after its intravenous administration (15mg/kg); b) determination of FSC concentrations in particular organ compartments. The elimination of FSC from blood is rapid ($t_{1/2}$=30min), thus the distribution study was carried out at 10 min after i.v. FSC administration (15% lower FSC levels compared to administered dose). The levels of FSC (μg/g of tissue) were severalfold higher in kidney and slightly increased in lung and liver compared to other organs (heart, pancreas, spleen, thymus) and the digestion tract tissues (esophagus, stomach, intestinal wall). There was shown that FSC was poorly distributed into the brain.

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REFERENCES

[1] Inoue H., *et al.* (2005) *Nat. Clin. Pract. Gastroenterol. Hepatol.* **2:**31–37.

THE CAPSULE ENDOSCOPY AS A DIAGNOSTIC TOOL FOR INDOMETACINE INDUCED GASTROINTESTINAL LESIONS

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In this pilot experiment, the capsule endoscopy "Olympus" [1] was utilized for the purpose of the perspective studies of absorption and biotransformation function of intestinal wall altered by toxicological agents. This technique allows non-invasive intraluminal scanning of particular intestinal segments *in vivo* (frequency=2 pictures/sec., life-cycle of camer electric source=8–9 hours). The experimental animals (n=5) were small adult pigs (30–36kg) as an omnivorous representative metabolically close to man. The clinical side effect of indometacine was utilized for inducing of gastrointestinal lesions. Indometacine (400 mg) was administrated orally for 10 consecutive days. The capsule microcamer was endoscopically inserted into duodenum 24 hours after the last dose of indometacine. Transmission antennae were fixed on the abdomen skin. Hence, the animals were kept under the general anaesthesia (thiopental intravenous administration) during the whole monitoring time. The image sequences scanned with microcamer enabled to judge the gradation of lesions and their quantity (red spots, erosions, ulcers) in the particular parts of small intestine. The evaluation of these experiments shown that applied "indometacine model" [2] evokes intestinal lesions with relatively low inter-individual variability regarding to development of morphological changes (various manifestation of inflammation in all animals with majority location from duodenum to middle segment of jejunum). On the other hand, the higher interindividual diference in their intensity was found. The pigs were sacrificed after the microcamer scanning termination and the collected tissue samples of stomach and intestinal wall were analysed with areal "confocal laser scanning endomicroscopy".

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REFERENCES:

[1] Korman L.Y., *et al.* (2005) *Endoscopy* **37:**951–959.
[2] Rainsford K.D., *et al.* (2003) *J Pharm. Pharmacol.* **55:**661–668.

GENETIC BACKGROUND OF CARCINOGENESIS IN THE THYROID GLAND – CURRENT STATUS OF KNOWLEDGE

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The process of carcinogenesis is permanently one of the most interesting and encompasses significant issues for researchers in different fields of medicine. The recent data on genetic factors involved in thyroid carcinogenesis are the subject of this presentation.

The review points to the role of some proteins, identified as important regulators of cell proliferation and DNA replication checkpoint, such as cyclin D1 and PIN1. These proteins are associated with cell transformation and cancer progression in different human neoplasms, including thyroid cancers. Molecular analysis has demonstrated that both *cyclin D1 gene* overexpression and aberrant *PIN1* (peptidylpropyl isomerase) *gene* expression are significant phenomena in many human cancers, including thyroid tumours and may play important role in carcinogenesis and metastasizing processes. We have assessed both *PIN1* and *cyclin D1* mRNA expression levels and their mutual relationship in benign (FA – follicular adenoma) and malignant lesions (PTC – papillary thyroid carcinoma, MTC – medullary thyroid carcinoma).

Our recent studies have been focused on mutations in the protooncogenes: *RAS, RET, Trk, MET,* and *BRAF*, as well as on the loss of heterozygosity (LOH) in short arm of chromosome 3 in thyroid neoplasms. It is worth recalling that previous studies performed at our laboratory have suggested the role of IGF-I in the pathogenesis and invasiveness of thyroid cancers.

Currently, we focus on hypermethylation studies. We use methylation-specific PCR to assess hypermethylation in several genes, including: *p16(INK4a), ARH1, MEST, KCNQ1* – involved in imprinting region (IR) and *RASSF1, SLC5A8, VHL, E-cadherin* (*ECAD*) – involved in nonimprinting region (NIR) of genome in thyroid lesions. Aberrant hypermethylation of CpG islands in the promoter region plays a causal role in the inactivation of various key genes involved in the cell cycle regulatory cascade and it can result in a loss of cell cycle control. Expression of many suppressor genes (*RB, p16, MASPIN, ARF*) is low or absent in most human cancers and it has been hypothesized that aberrant promoter methylation is the reason. In thyroid cancers, promoter methylation of numerous genes, e.g. genes encoding for thyrotropin receptor (*TSHR*), *ECAD,* sodium iodide symporter protein (*NIS*), is a well known observation. Methylation of *MASPIN gene* promoter is a common and early event in PTC development. Interestingly, expression of the recently cloned Pendred syndrome gene (*SLC26A4* or *PDS*) has been decreased or even absent in various thyroid tumors and it is suggested that epigenetic changes represent a new mechanism in altering the *SLC26A4 gene* function, in addition to genetic mutation in Pendred syndrome.

CHANGES IN IMMUNOLOGICAL PARAMETERS IN AIRCREWS OCCUPATIONALLY EXPOSED TO RADIATION AND STRESS

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The aim was to investigate changes in cellular and humoral immune response in male population occupationally long-term exposed to low-doses of ionizing radiation and stress.

Immunological and haematological parameters were examined in peripheral blood samples derived from 58 male pilots and crew members with cumulative exposures of 2–4 mSv/year (mean age 37 years) and from a reference (control) population of 40 male ground crews (mean age 32 years).

Immune function assays – proliferative response of lymphocytes to mitogens and antigens, phagocytic activity and respiratory burst of neutrophils were performed. Phenotypic analysis of peripheral blood leukocytes CD3, CD4, CD8, CD16+56, CD3+HLADR, CD19 were evaluated using flow cytometry. Concentrations of interleukins IL-10, IL-12 and IL-18 and soluble adhesion molecules sICAM-1 were determined.

Proliferative activity (ConA) of T-lymphocytes *in vitro* in cell cultures derived from pilots was significantly increased ($p>0.01$) in comparison with control group. T-dependent B-cell response to pokeweed mitogen and basal proliferation activity of lymphocytes showed in pilots similar significant changes ($p>0.001$) vs. control. On the other hand, phagocytic activity of monocytes ($p>0.05$), concentration of IL-12 ($p>0.01$) and IL-18 ($p>0.05$) were significantly suppressed in pilots in comparison with controls. Other monitored parameters did not differ significantly in two studied groups.

These results might suggest hypersensitivity and inflammatory status in occupationally exposed aircrews. Moreover, our findings indicate possible damage of cell-mediated immune response of T-lymphocytes in professionally exposed pilots.

Long-term exposure to radiation and stress indicate possible immunomodulatory effects in occupationally exposed aircrews. Changes in proliferative activity of lymphocytes and phagocytic activity in peripheral blood and findings of decreased levels of interleukins may indicate negative effects on the immune system of exposed pilots.

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EFFECT OF CARVEDILOL ON THE PRODUCTION OF REACTIVE OXYGEN SPECIES BY HL-60 CELLS

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Generation of reactive oxygen species (ROS) by phagocytes belongs to irreplaceable microbicidal tools of innate immunity. It has been reported that carvedilol *in vitro*, added simultaneously with the activator to the measuring mixture, inhibits ROS generation by activated blood leukocytes. The purpose of this study was to investigate the long-term effect of carvedilol on phagocytes. Human leukemia HL-60 cells were used as the model. Cells were cultivated in RPMI supplemented with 10% of fetal calf serum and only cells between passages 23 and 25 were used for the experiments. The cells were seeded at 96 well-plates at the amount of 60 000 cells per well. Than the cells were incubated in the presence of carvedilol or medium for 24h, 48h and 72h. Final concentrations of carvedilol were 0.0001–0.01 mM, these concentrations did not exhibit toxic effect on cells (measured using ATP test). The production of ROS was inhibited in cells treated by 0.01 mM carvedilol for 24 h and then activated with opsonized zymosan or phorbol myristate acetate. Lower carvedilol concetrations did not exert any effect at this time interval. On the other hand, the increase in ROS production was observed in cells after 48 h and 72 h co-incubation with carvedilol in all concetrations used. This effect was more pronounced in cells activated with phorbol myristate acetate in comparison with opsonized zymosan. The results suggest the different mechanisms of the effect of carvedilol on phagocytes activated with receptor binding or receptor by-passing stimuli.

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OXIDATIVE STRESS AND REPROTOXITY DURING CHRONIC EXPOSITION WITH HEAVY METALS IN RATS

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Oxidative stress occurs when the production of potentially destructive reactive oxygen species (ROS) exceeds the limits of the body natural antioxidant defence, resulting in cellular damage. Oxidative stress is a common pathology seen in approximately half of all infertilities in humans. We are estimated significant reprotoxic effects of longtime low-dose exposition to heavy metals in the rats. The purpose of this study was an evaluation of the relationships between antioxidant status and the reprotoxicity in rats exposed to low dose of lead, cadmium and mercury in drinking water (concentration 20 mg/l, 2 mg/l and 0.2 mg/l, respectively, these correspond to 200-times dose of NOAEL /No Observed Adverse Effect Level/). Significant decreases of TAS (Total Antioxidant Status), glutathione, catalase, GPx (glutathione peroxidase) and SOD (superoxide dismutase) corresponded with increase of reprotoxicity. Interestingly, cadmium exposure may in rats paradoxically improve some reproduction parameters as compared with lead and mercury exposure, and even untreated controls.

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ROLE OF OXYTOCIN IN RESPONSE TO ACUTE STRESS IN MALES

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Oxytocin (OT) belongs to a family of peptides that have been identified in all classes of vertebrates and many invertebrate species. It is synthesized in the hypothalamus and released into the bloodstream via the axon terminals in the posterior pituitary or neurohypophysis and it is important for maternal behavior (labor, lactation, social interaction). Although found in about equal concentrations in both sexes, the physiological importance of OT in males is still unclear.

The aim of this study was to investigate the role of OT in the behavioral responses to shaker stress, using oxytocin knockout mice (OTKO) and behavioral tools for assessment of locomotor activity in the open field, elevated plus maze, and levels of plasma corticosterone (Cort). We determined changes in locomotor activity in the open field and elevated plus maze, and levels of corticosterone after 15-minutes acute stress or 7 days of intermittent subchronic stress.

The decrease in basic movements and fine movements in the male OTKO –/– group was significantly lower compared to OTKO +/+ and total time in the periphery was just not significant ($p<0.09$). The interaction between stress and genotype was significant in basic movements ($p<0.05$). Similar to the open field test, a decrease in basic movements after stress was observed in the elevated plus maze and had the same pattern, with differences between OTKO –/– lower than OTKO +/+ males. Stress also increased total time in the closed arms in both genders and genotypes. Acute shaker stress significantly increased the levels of corticosterone in both genders, but the response to stress in females was 4-times bigger than in males. No changes were observed between genotypes after 7 days of subchronic intermittent stress, suggesting that oxytocin improved recovery and had an anxiolytic effect only after acute stress.

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INHIBITION OF SUPEROXIDE GENERATION AND MYELOPEROXIDASE RELEASE BY CARVEDILOL AFTER RECEPTOR AND NONRECEPTOR STIMULATION OF HUMAN NEUTROPHILS

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Activation of neutrophils induces generation of reactive oxygen species, including superoxide generation (SO), and release of granule enzymes, which participate not only in bactericidal mechanisms of these cells but also in possible tissue damage.

Carvedilol (CARV) is a non-selective beta-blocker, which has also alpha-blocking properties and antioxidant effects. This confers aditional therapeutical advantages when compared to classic beta-blockers.

We studied the effect of CARV [0.1–100μmol/l] on SO generation and myeloperoxidase (MPO) release from isolated human neutrophils after specific receptor activator – fMLP and nonreceptor – PMA and A23187 stimuli. CARV had no effect on SO generation and MPO release in nonstimulated cells. In the concentration 10 and 100 μmol/l, it significantly decreased both parameters after fMLP stimulation. Incubation of neutrophils with CARV [100μmol/l] caused significant inhibition of SO and MPO release induced by PMA and A23187. Wortmannin, a specific inhibitor of 1-phosphatidylinositol-3-kinase, inhibited significantly fMLP stimulated SO generation only. CARV [100μmol/l] with wortmannin [100μmol/l] decreased SO generation after the same stimulus.

Our results showing that CARV decreased SO generation and MPO release both by the membrane-operating stimulus fMLP (activation of G protein and in turn phospholipase C stimulation) and the membrane bypassing activators PMA (activating proteinkinase C) and A 23187, bypassing G protein and increasing the concentration of cytosolic free calcium, a powerful stimulus for cell activation. This fact indicates that the inhibitory effect of CARV may be attributed to its non-specific action , opening the possibility that, similarly to other lipophilic beta-adrenoceptors, it interferes with membrane structures. Inhibition of SO generation by CARV in stimulated human neutrophils not only reduces the toxicity caused by SO itself, but also prevents the formation of the higher reactive and toxic hydroxyl radical. Moreover, the toxicity and damage to surrounding tissues caused by MPO was also diminished.

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COMPARISON OF ACUTE TOXICITY OF SELECTED ANAESTHETICS TO JUVENILE AND EMBRYONIC STAGES OF *DANIO RERIO*

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The aim of this study is to compare acute toxicity of selected anaesthetics; clove oil and 2-phenoxyethanol, to juvenile and embryonic stages of zebrafish (*Danio rerio*).

Anaesthetics are used in aquaculture to prevent stress and mechanical damage to fish during handling or the treating of fish in breeding, blood sampling and other veterinary interventions. Clove oil and 2-phenoxyethanol

are used in the Czech Republic in water bath for short-term immobilization of fish.

Acute toxicity tests were performed on the aquarium fish *Danio rerio* which is considered as one of the model organisms most commonly used in toxicity testing. The main advantage of utilizing this popular aquarium fish is its easy availability, low price, and easy of rearing. The semistatic method according to OECD No. 203 (Fish acute toxicity test) was used in the acute toxicity tests with juvenile fish. Embryo toxicity tests were performed in zebrafish embryos (*D. rerio*) in compliance with the OECD No. 212 methodology (Embryo toxicity tests).

The results obtained (the number of dead individuals at particular test concentrations) were subjected to a probit analysis using the EKO-TOX 5.2 programme in order to determine LC50 clove oil and 2-phenoxyethanol values. The statistical significance of the difference between LC50 values in juvenile and embryonic stage of *D. rerio* was tested using the Mann-Whitney non-parametric test implemented in the Unistat 5.1 programme.

The LC50 values of these tests were compared. Acute toxicity values of clove oil for juvenile and embryonic stages seem to be similar for both stages. Acute toxicity values of 2-phenoxyethanol proved a higher sensitivity in the juvenile stage of *D. rerio*.

The work was realized with the support of MSM Project No. 6215712402 Veterinary Aspects of Foodstuff Safety and Quality.

NATIONAL GLP COMPLIANCE MONITORING PROGRAMME IN SLOVAK REPUBLIC

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To facilitate the mutual acceptance of test data generated for submission to Regulatory Authorities of the OECD and EU member countries, harmonization of the procedures adopted to monitor good laboratory practice (GLP) compliance as well as comparability of their quality and rigour, are essential. The aim of the GLP compliance monitoring programme is providing of detailed practical guidance to the member states on the structure, mechanism and procedures they should adopt so that these may be internationally acceptable.

National GLP compliance monitoring programme is the particular scheme established by Member State to monitor GLP compliance by test facilities within its territories by inspections and study audits.

National GLP monitoring authority is a body established within a Member state with responsibility for monitoring the GLP compliance of the test facilities within its territories.

SR is obliged by membership in EU to take over into legislation Commission Directive 1999/11/EC adapting to technical progress the principles of GLP as specified in Council Directive 87/18/EEC and Commission Directive 1999/12/EC adapting to technical progress for the second time the Annex to Council Directive 88/320/EEC on the inspection and verification of GLP and their codified versions 2004/9/EC and 2004/10/EC.

Prefaced obligations were in SR completed by issuing Act 163/2001 Coll. on chemical substances and chemical preparations, Act 140/1998 Coll on Medicinal products and Medical Devices and Order of the Ministry of Economy SR No. 65/2002 Coll.. Those Acts were supplemented by Act No. 95/2007 Coll., and Governmental Ordinance SR No. 298/2007 Coll. where are defined details about test facilities and inspectors monitoring compliance with principles of GLP.

SNAS was named by Act No. 95/2007 as the only competent authority in charge of the inspection of laboratories engaged in testing of chemicals and of the audit of studies carried out by them. Interdepartmental activities of monitoring authority are provided by its creation on SNAS basis.

All details about **Slovak National GLP compliance monitoring programme** can be found at www.snas.sk.

COMPARISON OF THE CYTOTOXIC/CYTOSTATIC EFFECT OF ETHYL-4-ISOTHIOCYANATO-BUTANOATE AND ITS N-ACETYLCYSTEINE AND GLUTATHIONE CONJUGATES

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Isothiocyanates ITCs from broccoli and other cruciferous vegetables have been identified as potent anticancer agents [1]. It has been showed that sulphoraphane (a major ITC in broccoli) and also its thiol conjugates has chemoprotective effect. Structural analogue of sulphoraphane ethyl 4-isothiocyanatobutanoate (E4IB) had been prepared fifteen years ago and its anticancer activity had been confirmed [2]. Molecular mechanism of anticancer effects of E4IB has been illuminated and recently a synergic effect of cisplatin combined with E4IB has been observed [3,4]. We have prepared conjugates of E4IB with N-acetylcysteine and glutathione and our study confirmed cytotoxic effects of these conjugates on human leukemia cells HL60 and its resistant subline HL-60/ADR. The cytotoxic effect of E4IB and its thiol conjugate against sensitive HL-60 line were similar (IC_{50} ranging from 1.4±0.8 to 3.0±0.6 μM). E4IB inhibited proliferation of HL-60/ADR resistant cells (IC_{50}=6.5 ± 1.5 μM) but its conjugates with thiols had weaker cytostatic effect (IC_{50}=13.1±0.8 μM, respect. 14.2±0.9), probably overproduction of MRP1 decreased intracellular concentration of substances. The utility of these substances as chemosensitizers of leukemia cells against doxorubicin has been investigated. Sensitive and resistant cells were co-treated with doxorubicin and tested substances. Our results showed that HL-60 cells were about 7-times more sensitive to the cytotoxic effects of doxorubicin in the presence of E-4IB and its conjugate with thiols. Isothiocyanate and its conjugate with thiol could bring benefit as chemosensitizers to

clinical chemotherapy by reducing the therapeutic doses of drugs.

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REFERENCES

[1] Hecht S.S. (1999) *J Nutr* **129:**768–774.
[2] Horakova K., *et al.* (1993) *Anticancer Drugs* **3:**369–75.
[3] Bodo J, *et al.* (2006) *Apoptosis* **8:**1299–310.
[4] Bodo J, *et al.* (2005) *J. Neoplasma* **52:**510–6.

THE DRUGS DURING LACTATION IN THE SCOPE OF THE CZECH TERATOLOGY INFORMATION SERVICE

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The benefits of breastfeeding are generally accepted. The problem appears if mother is exposed to drugs due to acute or chronic disease that has to be treated. Mothers have possibility to ask gyneacologists or pediatricians for solution their doubt if the medication can affect their safe breastfeeding. However, there is only too little information given, and the information about prescribing medications are frustrating and do not recommend lactation. Risk of drug usage for baby due to lactation is well assessed minimally in certain cases. In Czech Teratology Information Service (CZTIS) counselling we use these given information.

We have given advice in 50 cases inquiring the CZTIS about the risk of drug exposure during lactation. The most frequent queries were on chronic disease treatment following the drug exposure during pregnancy. Remaining cases were associated with acute infections. Mothers suffered from idiopathic bowel disease and psychiatric patients want to be informed before delivery about possibility to breastfeed their babies. Treatment of epilepsy, another frequent disease, is associated with better level of knowledge of both, neurologists and patients. Breastfeeding is recommended according to management in care of epileptic women.

In our counselling we consider the factors which are involved in drug transfer in the milk and mechanisms and steps of transfer as well. We follow the classification of drugs during lactation by their effect on infants: absolutely contraindicated, temporary cessation of breastfeeding, drugs of special concern and drugs compatible with breastfeeding.

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USE SEQUENCE INJECTION ANALYSIS AND IN-ELECTRODE COULOMETRIC TITRATION TO DETERMINATE SOME IMPURITIES IN WATER

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The expanded use of ozonation for the disinfection of drinking water has resulted in a growing concern over disinfection byproducts of ozonation. One such disinfection byproduct of ozonation that has been identified is bromate. Bromate has been classified by the International Agency for Research on Cancer (IARC) as having sufficient evidence of carcinogenicity in laboratory animals. The USEPA has proposed a maximum contaminant level (MCL) of 10 $\mu g.dm^{-3}$ for bromate [1].

Method is based in electrochemical reduction of bromate ions by bromide to bromine and determinated by chronopotenciometry.

$$BrO_3^- + 5\ Br^- + 6\ H^+ = 3\ Br_2 + 3\ H_2O$$
$$3\ Br_2 + 6\ e^- = 6\ Br^-$$

Computer controlled signal processing enabling measure concentrations down to low $\mu g.dm^{-3}$.

The determination of bromate by new coulometric method in tap water samples is simple, accurate, relatively fast (20–30 min preparation, 1–2 min measuring). We can measure low concentration in the linear range from 0.5 $\mu g.dm^{-3}$ to 50 $\mu g.dm^{-3}$. The accurance of measurements was checked by method addition of standard. Recovery of bromate was in range 96–98%. The developed method should renew and compete the ion chromatography with "postcolumn" derivatization, but is significantly cheaper and faster.

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REFERENCES

[1] von Gunten U., *et. al.* (1996) *Journal AWWA*

TWELVE DIFFERENT HI-6 SALTS AND THEIR POTENCY TO REACTIVATE CYCLOSARIN INHIBITED ACHE *IN VITRO*

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As it is generally known, different anions of pharmaceutical preparations are generally developed to achieve better pharmacological effect through their adsorption phase. In this article, reactivation potency of twelve salts (sulfate, chloride, acetate, bromide, phosphate, mesylate, tartarate, iodide, malonate, salicylate, maleinate, tosylate) of bisquaternary acetylcholinesterase reactivator HI-6 was tested to elucidate that chemically different anions have no effect on the reactivation of nerve agent-inhibited acetylcholinesterase. For this purpose, cyclosarin was taken as the appropriate member of the nerve agent family. It was found that the use of different salts has no effect on the reactivation potency.

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SCREENING AND SEMIQUANTITATIVE ANALYSIS OF DRUGS AND DRUGS OF ABUSE IN HUMAN SERUM SAMPLES USING GAS CHROMATOGRAPHY-MASS SPECTROMETRY

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The object of the study was to develop a gas chromatography-mass spectrometry GC-MS method for screening and semiquantification of drugs and drugs of abuse in human serum. GC-MS possesses high potential in systematic toxicological analysis because of its separation power of GC as well as the selectivity of detection of MS.

After liquid-liquid extraction of 2 ml bovine serum spiked with common drugs and drugs of abuse codeine, morphine, ephedrine, 3,4-methylenedioxymethamphetamine, tramadol, dothiepin, cocaine, mirtazapine, clomipramine, alprazolam, zolpidem, clozapine, amitriptyline, citalopram, diazepam, levomepromazine, bromazepam, phenobarbital, guaifenesin and internal standards trimipramine d_3 and hexobarbital (at pH=8.0–9.0 basic and neutral analytes were extracted with 4 mL of ethylacetate:1–chlorbutane:cylohexane (3:1:1 v/v/v), at pH=2.0 acidic analytes were extracted with 4 mL of ethylacetate:toluene (4:1 v/v)). The analytes and internal standards were separated on HP-5ms 30 m × 0.25 mm i.d. with 0.25 μm film thickness. The compound were screened for and identified using a Finnigan MAT MAGNUM ion trap GC-MS with Varian 3400 GC fitted with SPI injector and A200S autosampler operated in full scan mode. The detection of compounds with active hydrogen atoms in the molecular structure were performed after silylation. Validation included evaluation of recovery, linearity and repeatability.

The analytes were sufficiently separated and sensitively detected. The calibration curves were linear in concentration range 0.025–2.0 μg/mL with correlation coefficients exceeding 0.99. The limit of quantification for all drugs was 0.05 μg/mL. The repeatabilites expressed as relative standard deviations were lower then 10%. The extraction efficiency were tested on concentration levels 0.05, 0.1 and 0.5 μg/mL and establised in range 72–98%.

In this study, GC-MS method is presented for screening for as well as identification and semiquantification of drugs and drugs of abuse in human serum and the application of the described assay was tested by analysis of real samples from clinical and forensic toxicology cases.

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PREPARATION OF APO-CYTOCHROME b_5 UTILIZING APO-MYOGLOBIN

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Cytochrome b_5, a component of endoplasmic reticulum membrane, is a heme protein with molecular weight of 16 800. It is composed of two functional domains, a soluble heme-containing core, and a short hydrophobic C-terminal tail, which anchors the protein into the microsomal membrane. Cytochrome b_5 has been shown to stimulate, inhibit or have no effect on cytochrome P450 (CYP)-mediated reactions. There are two major theories explaining this effect: (i) direct electron transfer from cytochrome b_5 to CYP and (ii) conformational effects of cytochrome b_5 on CYP without contribution of electron transfer. 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 by this system. To study the mechanism of this modulation, not only the effect of native cytochrome b_5, but also that of the apo-cytochrome b_5 (lacking the electron transferring cofactor – heme) on ellipticine oxidation is necessary to be evaluated.

In order to prepare the pure apo-cytochrome b_5, the heme cofactor has to be gently and efficiently removed without altering the native protein conformation. We utilized another protein eliciting high affinity toward the heme – the apo-myoglobin from the equine skeletal muscle. In the first step, we extracted heme moiety from the – native myoglobin by butanone extraction. Than the prepared apo-myoglobin was incubated with the cytochrome b_5 and heme transfer was monitored as a shift of absorption spectrum from 413 to 409 nm. Apo-cytochrome b_5 was separated from the myoglobin by two-step ionex chromatography.

Moreover, we employed a mutant of myoglobin (SWMb_H64Y/V68F), which has even higher affinity to heme than the wild type myoglobin. H64Y/V68F mutant of myoglobin was produced by heterologous expression in *Escherichia coli* BL21 (DE3) Codon Plus RIPL cells. Protein was purified by CM-cellulose column chromatography and its apo-form was again prepared by butanone extraction. Apo-cytochrome b_5 was separated from the mutant myoglobin by two-step ionex chromatography.

The experiments investigating the effect of the purified native cytochrome b_5 and apo-cytochrome b_5 on oxidation of ellipticine by CYP1A1 and 1A2 are under way in our laboratory.

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COMPARISON OF GLUCURONIDATION PROCESSES IN THE MAN, RAT, PIG, MONKEY AND DOG

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Glucuronidation is a major phase II pathway of xenobiotic biotransformation in mammalian species. The reaction is catalysed by UDP-glucuronosyltransferases

(UGTs), which are located in the endoplasmatic reticulum of liver and other tissues. Till now, 15 human UGTs are known [1]. Unfortunately, little has been known about glucuronidation in other mammals, although the activity of UGTs in liver is an integral part of toxicological research for xenobiotic chemicals. The aim of this work is to compare the activity of UGTs towards p-nitrophenol (pNF), 4-methylumbelliferon (4-MU) and silybin in human, rat, pig, monkey (*Maccaca rhesus*) and dog (Beagle), using liver microsomal fraction of the species mentioned. To determine the formation of glucuronides, we have used an HPLC method with UV detection [2]. In case of planar phenol derivatives, such as pNF and 4-MU we have not reported any significant difference in the rate of formation of pNF β-D-glucuronide and 4-MU β-D-glucuronide across tested species. To the contrary, more complex molecules, such as silybin, allow formation of glucuronides also in more positions (in silybin, in positions 7, 20α and 20β). We have reported not only different rates of formation of silybin glucuronides but also differences in relative amounts of metabolites in positions 7, 20α and 20β. For example, in the man, form of 7 β-D-glucuronide is predominant, whereas in case of the pig, the relative amount of silybin glucuronides is relatively equal.

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REFERENCES

[1] Parkinson A. (2001) *Casarett & Doull's Toxicology* ***6th ed:***133–224.
[2] Hanioka N. *et al.*(2001) *J Pharm Biomed Anal* **25:**65–75.

CHROMOSOMAL DAMAGE AND POLYMORPHISM IN GENES DETOXIFICATION ENZYMES *GSTM1*, *GSTT1* AND *GSTP1* IN EPOXIDES EXPOSED WORKERS

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Workers in boot – and – shoe industry have been chronically exposed to epoxide with potential consequences on chromosomal integrity. Our study is focused on the level of chromosomal aberrations as well as on the tentative modulating role of polymorphisms in detoxification genes EPHX, GSTM1, GSTT1 and GSTP1 on chromosomal damage.

Benzo(a)pyrene-7,8-epoxide is environmental carcinogenic compound reacting with DNA and/or proteins, giving rise to specific adducts, but could also lead to a significant increase in the levels of biomarkers associated with DNA lesion (e.g. DNA adducts, sister chromatid exchanges (SCEs), chromosomal aberrations (CAs) micronuclei (MN)). Glutathione S-transferases (GSTs) are a superfamily of polymorfic enzymes involved in conjugation of reactive intermediates to soluble glutathione forms, playing an important role in the detoxification of endogenous and exogenous toxicants. Polymorfic genes *GSTM1*, *GSTT1* and *GSTP1* are also involved in the detoxification of hydrocarbon diol-epoxides. One of the *GSTP1* polymorphisms is result of a base substitution (A→G), which leads to aminoacid substitution (codon 105, Isoleucine→Valine). This aminoacid substitution, in the GSTP1 binding site, modifies its catalytic activity. *GSTM1* and *GSTT1* polymorphisms are characterised by the deletion of part of the gene, which leads to absence of activity. Polymorphisms *GSTs* genes have been associated with increased chromosomal damage.

The present study was performed on 40 workers that have been exposed to epoxides for 6.2±2.31 years, and 30 control individuals. Conventional cytogenetic analysis was employed for detection of CAs. *GSTM1, GSTT1 and GSTP1* polymorphisms were assayed for by polymerase chain reaction (PCR) and restriction fragment length polymorphisms (PCR-RFLP).

Results of our study show modulating effect of individual susceptibility on frequency of chromosomal aberrations.

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PATHOGENESIS OF FAMILIAR ADENOMATOUS POLYPOSIS (FAP)

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The colorectal carcinomas (CRC), especially their hereditary forms, represent an excellent model for studying of the genetic alterations leading to tumorigenesis. The cancer formation is a multistep process, which assumes that the accumulation of mutations in at least 3–4 genes is required for initiation and progression of malignant growth.

The CRC is the second leading cause of cancer deaths in Europe. More than 20% of all cases are family-associated and half of these family-associated CRC count for familial adenomatous polyposis (FAP). FAP is an autosomal dominant inherited disease with incidence of 1:5 000. Patients with FAP predisposition are heterozygous for adenomatous polyposis coli (APC) gene. Inactivation of the second APC allele, which takes place prior accumulation of mutations in other genes (e.g. K-ras, TP53) leads to the loss of its tumor suppressor activity and to initiation of tumorigenesis.

Penetration of FAP is approximately 95%. The main clinical symptom of disease is the presence of polyps (100–1000) through the colon. The mutations are spread all through the APC gene; 70% of all mutations have been found in the largest 15th exon resulted in truncation and thus malfunctioning of the APC protein. Presymptomatic FAP diagnostics based on the detection of the APC gene mutations allow early detection of the disease.

The frameshift mutations in codon 1309 and 1060 are the most frequent mutations found in Slovak population and represent about 25% of all detected ones. These mutations, including nonsense mutations in codon 1272, have very severe prognosis. The nonsense mutations in codons 282, 213 and frameshift mutation in codon 753 are not always clinically expressed. Therefore, molecular diagnostic is for these patients vital. It is supposed that the polymorphisms, frequently found in APC gene, are not diagnostically important.

In our laboratory, we were tested members of 162 families suspected from adenomatous polyposis. The broad spectrum of mutations was detected in 42 families. The patients with detected mutation were integrated into therapeutic program.

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IDENTIFICATION AND DETERMINATION OF THE FLY AGARICS TOXINS BY LC-MS

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Over the past twenty years, the expansion of the drug scene in the Czech Republic has been characterized by an expressive increase of drugs abuse, especially of cannabinoids and amphetamines. In the last years, substances contained in psychotropic mushrooms, such as toxins of *Amanita muscaria* and *Amanita pantherina*, have also occured among the drugs-of-abuse. The hazard to the consumers of these mushrooms is posed by occurring neurotoxic symptoms. Although intoxications through these mushrooms are rarely lethal, it is important to determine them soon and initiate a medical treatment.

The psychotropic effects of *Amanita muscaria* and *Amanita pantherina* are caused by isoxazole derivates – ibotenic acid and muscimol and by muscarine. Variable concentration of these toxins significantly increases the risk connected with the abuse of these mushrooms.

At present, the diagnosis of these mushroom poisonings is almost entirely determined by microscopic examinations, due to the lack of a suitable analytical method for identification and determination of the toxins from urine, blood or gastric content.

The aim of the project is to elaborate and introduce an objective and validated analytical method for a rapid and reliable diagnosis of intoxications through these mushroom toxins. The LC-MS method was chosen in regard of the character of these toxins. In this presentation the authors discuss the conditions for isolating these toxins from biological material and the optimalization of chromatographic conditions of LC-MS method with respect to its possible utilization in routine toxicological practice.

This work is supported by the Ministry of Education of the Czech Republic (MSM 6198959216)

ROLE OF CYTOCHROME CYP3A4 IN BIOTRANSFORMATION OF *7H*-DIBENZO[*C,G*]CARBAZOLE AND ITS TISSUE SPECIFIC DERIVATIVES

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Cytochromes P450 (CYP450) is a superfamily of hemoproteins found in bacteria, fungi, plants and animals. CYP450s are involved in the biotransformation of various structurally-diverse endogenous and exogenous compounds, including drugs, toxicants and procarcinogens. It is supposed that differences in P450s distribution among tissues and organs of mammalian organism might underlie the tissue specificity of chemical carcinogens.

Cytochrome P4503A (CYP3A) subfamily is one of the most important drug metabolizing families in humans. Moreover, CYP3A4, the most abundantly expressed CYP in human liver is involved in the metabolism of more than 50% of clinically used drugs, including procarcinogens.

The aim of this study was to evaluate the role of CYP3A4 in biotransformation of 7*H*-dibenzo[*c,g*] carbazole (DBC) and its tissue and organ specific derivatives. While DBC, a ubiquitous environmental pollutant, possesses both the hepatocarcinogenic and sarcomagenic activities, its two methyl derivatives, *5,9*-dimethyldibenzo[*c,g*]carbazole (diMeDBC), an organ specific carcinogen (hepatocarcinogen), and *N*-methyldibenzo[*c,g*]carbazole (*N*-MeDBC), a tissue specific carcinogen (sarcomagen), manifested specific tropism for the liver and skin, respectively.

The cytotoxic and genotoxic activity of these carcinogens were evaluated in the genetically engineered Chinese hamster V79 cell line (V79MZh3A4) with stable expression of human CYP3A4. Benzo[*a*]pyrene (BaP), a well-known sarcomagenic PAH, and aflatoxin B1, a proven hepatocarcinogen, were used as positive controls.

A dose-dependent decreased in colony forming ability (CFA) was detected in V79MZh3A4 cells exposed to hepatocarcinogens, DBC and diMeDBC. In contrast, *N*-MeDBC, a tissue specific sarcomagen, did not change substantially cell viability. A slight but statistically significant ($p<0.05$) increased frequency of gene mutations was determined in DBC- and partially in MeDBC-treated cells; however, cell exposure to diMeDBC did not result in an elevated level of 6-TG^r mutations compared to control. The sequence analysis of the coding region of *HPRT* gene revealed large deletions induced by reactive intermediates formed due to activation of diMeDBC *via* CYP3A4. Based on these data we suppose that the human CYP3A4 is involved in biotransformation of these tissue specific carcinogens. Differences in the cytotoxic and genotoxic effects induced by particular compound might indicate that different metabolites/quantity of

metabolites are formed due to CYP3A4 activation in dependence on the chemical structure of the agents.

This study was supported by VEGA grant No. 2/6063/27.

RAT AND HUMAN CYTOCHROMES P450 OXIDIZE 3-AMINOBENZANTHRONE, THE HUMAN METABOLITE OF CARCINOGENIC 3-NITROBENZANTHRONE

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The aromatic nitroketone 3-nitrobenzanthrone (3-NBA) is one of the most potent mutagens and suspected human carcinogen identified in diesel exhaust, ambient air particulate matter, in surface soil and rainwater. The main metabolite of 3-NBA, 3-aminobenzanthrone (3-ABA), was recently detected in the urine of smoking and nonsmoking salt mining workers occupationally exposed to diesel emissions, demonstrating that exposure to 3-NBA can be significant and is detectable. Here, we study the metabolism of 3-ABA *in vitro*, in order to characterize the 3-ABA metabolites as well as cytochromes P450 responsible for their formation. We recently showed that principal enzymes forming DNA adducts from 3-ABA in livers are cytochromes P450 (CYP), especially CYP1A1 and 1A2.

Oxidation of 3-ABA by hepatic microsomes of rats pre-treated with different inducers of CYPs in the presence or absence of CYP inhibitors was studied. Rat hepatic cytochromes P450 in microsomes oxidize 3-ABA up to three metabolites. These metabolites were separated by HPLC as distinguish product peaks. Using co-chromatography with synthetic standards, two of them were identified to be oxidative metabolites of 3-ABA, *N*-hydroxy-3-ABA (r.t. of 6.5 min) and 3-NBA (r.t. of 25 min). Structure of third metabolite, eluted with the retention time of 18 minutes, assignend as M18, remains to be characterized. Oxidation of 3-ABA by microsomes isolated from livers of rats treated with β-naphthoflavone (β-NF), an inducer of CYP1A, phenobarbital (PB), an inducer of CYP2B, and by those of control (untreated) rats was analyzed.

The most effective oxidation of 3-ABA was detected using PB-microsomes, followed by β-NF-microsomes. These results indicated the major role of CYP2B and 1A in 3-ABA oxidation. Influence of selective CYPs inhibitors on the 3-ABA oxidation was confirmed. The values of inhibition constant (IC_{50}) for several CYPs inhibitors were determined. Of the inhibitors, diamantane was the most effective inhibitor of 3-ABA oxidation. The results suggest the participation of several CYP enzymes of the microsomal systems (mainly CYP2B and 1A1/2) in formation of individual metabolites of 3-ABA. Using Supersomes, microsomes containing human and rat recombinant CYP enzymes, participation of individual CYPs in 3-ABA oxidation was confirmed.

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MIXTURES OF SELECTED ENDOCRINE DISRUPTORS: *IN VITRO* STUDY ON THEIR EFFECTS ON OVARIAN FOLLICULAR CELLS PHYSIOLOGY

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Environmental chemicals termed as endocrine disruptors have raised a significant concern as a potential hazard for reproduction. Phenol and phthalate derivatives, such as bisphenol A (BPA), chlormethyl phenol (CMP), and benzyl butyl phthalate (BBP), are used as plasticizers and stabilizers of plastics, and thus present in everyday-use products (food covers, cosmetics). The present study was aimed to analyze the effects of selected phenol-phthalate binary or ternary mixtures, respectively, on progesterone production by ovarian granulosa cells (GC) and on the processes important for the release of fertilizable ovum: expansion of oocyte-cumulus complex (OCC) and oocyte nuclear maturation.

OCC and GC were isolated from porcine ovarian preovulatory follicles. After 24 h incubation of OCC with the tested mixtures (10^{-10} to10^{-4} M), FSH-induced cumulus expansion was assessed stereomicroscopically and hyaluronic acid synthesis (HA) by cumulus cells was measured radiometrically. After 44 h, nuclear maturation of orcein-stained oocytes was analyzed. After 72 h incubation with the mixtures, either basal or FSH-stimulated progesterone (P) production by GC was measured in the culture media by radioimmunoassay.

Both binary (BPA+BBP) and ternary (BPA+CMP+BBP) phenol-phthalate mixtures suppressed expansion of cumulus cells and inhibited meiotic maturation of the oocytes at the highest concentration. The results indicate that the mixtures altered basal as well as gonadotropin-stimulated progesterone production by GC. In dependence of composition, the mixtures exerted more significant changes on progesterone synthesis in comparison with individual compounds.

The results suggest importance to study not only the action of individual endocrine disruptors but also the mixtures of these chemicals as they might be diverse. Moreover, this model bears similarities to a real life, since humans and wildlife accumulate a large number of different environmental agents that might alter processes necessary for successful ovulation and fertilization.

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EFFECTS OF METRIBUZINE SUBCHRONIC EXPOSURE ON COMMON CARP (*CYPRINUS CARPIO*)

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Consequences of aquatic environment pollution pose an intensively discussed problem of today. Sublethal exposure to a pesticide can cause disturbance of normal metabolism in fish. The aim of our study was to examine disruptive potential of low concentrations of triazine herbicide metribuzine on common carp *Cyprinus carpio*, observed on subchronic condition. It was tested in the form of Sencor 70 WG pesticide, the active substance of which is metribuzine in the amount of 700 g/kg. Haematological and biochemical indices were determined in one year old carps after 28 days of exposure to 1.75 mg/l and 0.175 mg/l metribuzine. Cytochrom P450 concentration and ethoxyresorufin-O-deethylase activity were estimated spectrofotometrically and spectrofluorimetrically, respectively. Hepatic vitellogenin level was evaluated by direct ELISA. Hematocrit, red blood cell count and hemoglobin content were found to be increased in both treated groups. However, there was significant difference ($p<0.05$) in 1.75 mg/l metribuzine for hematocrit and red blood cell count only. Although no significant differences were observed in total protein, albumin, triglycerids and glucose levels and in alanin aminotransferase activity, these were found to deplete slightly in both exposure concentrations. Lactate content and sodium ion were elevated insignificantly in 1.75 and 0.175 mg/l metribuzine. We didn't find out endocrine disruption of reproduction measurable by male fish vitellogenin induction. Cytochrom P450 concentration and EROD activity were not influenced in the study. Metribuzine did not affect body weight or rates of hepatosomatic index in exposed fish. No mortality was shown in test concentrations.

The conclusion was drawn that sublethal metribuzine pollution may have adverse impacts on haematological parameters in common carp.

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CHARACTERIZATION OF ADDUCT GENERATED BY ELLIPTICINE METABOLITE 13-HYDROXYELLIPTICINE WITH DEOXYGUANOSINE IN DNA

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Ellipticine is an alkaloid exhibiting potent antineoplastic activities. This agent and some its derivates are used in the therapy of breast cancer and leukemia and have multiple cellular targets. Recently, we found that ellipticine forms covalent DNA adducts *in vitro* and *in vivo* and that the formation of the major adduct is dependent on the activation of ellipticine by cytochromes P450 (CYP) and peroxidases. 13-Hydroxyellipticine formed by CYP3A4 as the predominant metabolite in human livers was identified to be bound to deoxyguanosine in DNA, generating the major DNA adduct. To characterize this adduct, we investigated its formation *in vitro* and the effect of pH and the phase II biotransformation enzymes, sulfotransferases (SULTs) and *N,O*-acetyltransferases (NATs) on efficiency of 13-hydroxyellipticine to form this DNA adduct. 13-Hydroxyellipticine incubated with DNA (or with deoxyguanosine) *in vitro* generates the major deoxyguanosine adduct, which was detected and quantified by the nuclease P1 version of the ^{32}P-poslabeling technique. HPLC was used to isolate the adduct formed in the reaction mixture from deoxyguanosine and 13-hydroxyellipticine. The levels of this DNA adduct were significantly increased by presence of the SULT conjugation enzymes expressed in the target tumors for ellipticine action (human breast cancer). Likewise, NAT1 and NAT2 stimulated the formation of the DNA adducts. By such stimulation of the ellipticine-DNA adduct 1 formation; the pharmacological efficiency of ellipticine is increased. The results shown in this study allow us to proposed the mechanism of the reaction responsible for formation of deoxyguanosine adduct 1 in DNA, found *in vitro* from either 13-hydroxyellipticine or formed from ellipticine by CYPs and *in vivo* in rats treated with this anticancer drug. We predict that ellipticine is bound to deoxyguanosine by its 5-methyl group, which is activated after hydroxylation due to CYP-mediated oxidation to alcohol (13-hydroxyellipticine). The study targeted to confirm this suggestion is underway in our laboratory.

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TARGETED TRANSPORT OF DRUG LOADED IN NANOPARTICLES: MAGNETIC FLUID, NANOPARTICLES TOXICITY AND DISTRIBUTION

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Development of efficient drug delivery systems has attracted great attention during last two decades. Drug delivery from carrier systems can avoid unwanted effects of the free drugs because of controlled biodistribution. The use of nanoparticles of biodegradable polymers as an alternative administration of anticancer drugs has advantages in enhancing therapeutic efficacy and reduces systemic side effects.

PLGA (poly (D, L-lactic-*co*-glycolic) acid) nanospheres (NPs) loaded with magnetic fluid (MFPEG) and anticancer drug Taxol prepared by the nanoprecipitation method

were used. We evaluated the safety of individual components of this delivery system. Toxicity studies were conducted in healthy ICR male mice, whereas efficacy *in vivo* was performed in B16 melanoma model in C 57BL/6 mice.

LD_{50} value of PLGA nanoparticles determined using Up and Down method (OECD 425) was 221 mg /kg, of magnetic fluid 396 mg Fe_3O_4/kg. The toxicity of NPs with magnetic fluid PLGA/MF PEG (100 mg PLGA/50 mg Fe_3O_4/10 ml) was determined to be in the range of 174–198 mg/kg (expressed in concentration of PLGA). Taxol loaded magnetic PLGA NPs (100mg PLGA/50 mg Fe_3O_4/5mg Taxol) caused symptoms of toxicity at the dose of 90 mg/kg.

The distribution of taxol in mice after single and repeated dose application of taxol loaded PLGA nanosheres in comparison with free taxol formulation and the effect of external magnetic field on the biodistribution of nanoparicles loaded with magnetic fluid were evaluated. The aim of these experiments was to study the behaviour of the magnetic fluid after injection into blood vessels, and the changes of biodistribution caused by the implantation of small magnet and in external magnetic field.

The efficacy of drug release *in vivo* and the efficacy of external magnetic field was demonstrated in preliminary *in vivo* efficacy model in mice. The enrichment of iron in surround tissues was confirmed by histopathological analysis (only with use of external magnetic field) and through inhibitory effect demonstrated in reduction mass of B 16 melanoma.

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THE RELATIONSHIP BETWEEN CHROMOSOMAL ABERRATIONS AND POLYMORPHISMS OF *GSTM1, GSTT1, GSSP1* AND *EPHX1* GENES IN MEDICAL WORKERS EXPOSED TO VOLATILE ANESTHETICS

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The aim of present study was the evaluation of genotoxic effect of volatile anaesthetics on occupationally long-term exposed medical workers. Simultaneously we focused on determination of relationship between total chromosomal aberrations (CAs), and specific types i.e chromosome (CSA) and chromatide (CTA) type in exposed and control groups. Individual susceptibility to chromosomal damages was related to polymorphisms of *GSTM1*, *GSTP1*, *GSTT1* and *EPHX1* genes.

The exposed group consists of 76 persons from Clinic of anesthesiology and intensive medicine Medical Faculty Hospital (MFH) in Martin and Central Military Hospital in Ružomberok. The control group contains 76 persons unexposed to anaesthetics. CAs levels were detected in peripheral blood lymphocytes using cytogenetic analysis, single nucleotide polymorphisms using PCR-RFLP method.

Significantly higher frequency (Mann-Whitney U-test, $p<0.0001$) of CAs and CSAs were detected in exposed group in comparison to control (CAs 2.49% vs. 1.45%; CSAs 1.89% vs. 0.75%, respectively). On the contrary in exposed group was significantly lower frequency of the CTAs ($p=0.04$) as compared to control (0.61% vs.0.70%, respectively).

In exposed individuals with null variant was detected higher frequency of CAs in comparison to plus variant (3.00% vs. 2.88% respectively) of the gene *GSTM1*. In the gene *GSTP1* was higher frequency of CAs in individuals with wild type (AA) alleles in comparison to heterozygous (AG) 3.00% vs. 2.82%. In gene *GSTT1* was higher frequency of CAs in individuals with null variant (3.71%) in comparison to plus variant (2.79%, $p<0.05$). In the exposed group was detected the highest frequency of CAs in individuals with high activity in the gene *EPHX1* (3.50%) in comparison to middle and low activity (2.54% vs. 3.08%, respectively).

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PROGRESS OF ACETYLCHOLINESTERASE REACTIVATORS IN THE CZECH REPUBLIC

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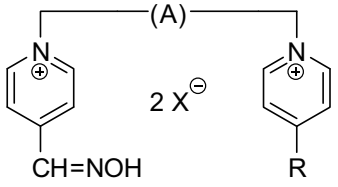
The reactivators of acetylcholinesterase (AChE, EC 3.1.1.7) are very important components in the treatment of intoxications caused by organophosphate inhibitors such as nerve agents and pesticides [1]. These inhibitors covalently bind to active site of mentioned enzyme and irreversibly inhibit its activity. The reactivator breaks the inhibitor-enzyme covalent bond and restores its activity. Unfortunately, there is no reactivator applicable for every type of inhibitor; it means that every structural change in the molecule of inhibitor needs a specific structure of the reactivator [2].



Several series of AChE reactivators have been prepared in last 5 years in Czech Republic. The key structural features have changed during this period. Namely, the bisquaternary compounds with two hydroxyiminomethyl (oxime) groups were turned into mono-oxime compounds, whereas the second heteroarenium ring carried another moiety. This second non-oxime moiety

highly influenced an activity and also toxicity of the reactivator.

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REFERENCES

[1] Bajgar J. (2004) *Adv. Clin. Chem.* **38:**151.
[2] Marrs T.C. (1993) *Pharmacol. Therapeut.* **58:**51.

THE INFLUENCE OF THE OROFACIAL CLEFT AND OF SOME TETRATOGENES ON THE ANTHROPOMETRIC PARAMETERS OF THE NEWBORNS WITH CLEFTS

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The purpose of this study was to acquire the antropometrical characteristics of the newborns with the orofacial cleft and to compare them with healthy newborns. The study also tries to identify the frequency of the occurrence of contagious diseases of mothers which bore the children with orofacial cleft during their pregnancy. Teratogene factors like age of the mother, smoking, alcohol and coffee drinking, stress during the pregnancy were taken into the consideration.

The study analyzed records of 129 newborns with orofacial cleft born from 1983 to 2007 between 35th and 42nd week of pregnancy which were supervised in the orofacial cleft clinic in Bratislava. The data included: the weight (g), height (cm), head and chest perimeter, of newborns. These results were compared with 159 healthy newborns born in 2007 of which 70 were girls and 89 boys. These data were acquired from the maternity ward of the „Nemocnica s poliklinikou, Ružinovská 6" hospital in Bratislava, Slovakia.

The age of mothers of newborns with orofacial cleft ranged from 17 to 40 years. During the pregnancy 54% of them have experienced cold, 51.5% overcame influenza, 23.6% suffered from herpes, 19.4% overcame the infection of the urinary system. Cca. 24% of women reported another kind of contagious diseases. 22.6% of mothers did not suffer from any contagious disease during the pregnancy, but 32.7% of women experienced vagina hemorrhage. As much as 19.4% of mothers regularly smoked, 55.2% drunk coffee, 23.6% drunk alcohol and 29.7% of mothers were exposed to stress during the pregnancy.

The acquired anthropometric parameters were compared with the controlling group. Statistically, these differences were insignificant.

Smoking during the pregnancy was found to have a big influence on the weight of the child (coefficient of correlation was r=0.313; p=0.05). This result comforms with the general scientific knowledge. Newborns whose mothers were drinking alcohol during the pregnancy were found to have smaller head perimeter than average.

According to the research results, we can claim, that orofacial clefts had no significant influence on the observed anthropometric parameters of the newborns. The minor differences in the anthropometric characteristics of the nurslings can be ascribed to constricted food intake as well as to increased morbidness of babies with orofacial cleft.

AN EVALUATION OF THE BIOLOGICAL AND TOXICOLOGICAL PROPERTIES OF *CYNARA CARDUNCULUS* L. AND *CHELIDONIUM MAJUS* L. EXTRACTS

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Latterly, in the period of integration of the alternative and conventional cancer treatment modalities of chemotherapy, high amounts of natural compounds isolated mostly from plants with various biological activities are of great importance. In accordance with a principle for the selecting new effective drugs for use in anticancer chemotherapy regimens, we decided to examine the eligibility of two extracts isolated from plants *Cynara cardunculus* L. and *Chelidonium majus* L. for potential cancer chemotherapy. Extracts were first tested *in vitro* for their cytototoxicity against mammalian cancer cell lines and because they responded in dose-dependent fashion at concentrations used we looked at their other potential biological activities, like apoptosis inducing and antioxidant property. We were evaluating also their main components characteristics. Results are of good promise. So, a rational use of phytochemicals is based not only on the assessment of their efficacy and safety but also on understanding their mechanisms of action. Therefore, such plants or plant extracts should be investigated to comprehend better their properties, safety and effectiveness.

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REDOX CYCLING IN METABOLISM OF CARCINOGENIC *o*-ANISIDINE BY RAT AND RABBIT HEPATIC MICROSOMES

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We investigated metabolic pathways of an industrial pollutant and a bladder carcinogen for rodents (for

summary see [1]), 2-methoxyaniline (*o*-anisidine). The International Agency for Research on Cancer has classified *o*-anisidine as a group 2B carcinogen, which is possibly carcinogenic to humans. Besides its carcinogenity, it exhibits other effects, including hematologic changes, anemia and nephrotoxicity. *o*-Anisidine is used as an intermediate in manufacturing of azo and naphtol pigments and dyes, which are used for printing and for paper and textile dyeing. Such a wide use of this aromatic amine could result in occupational exposure. This carcinogen is also a constituent of cigarette smoke and was also found in human urine in general population. This strongly suggests that *o*-anisidine ranks not only among occupational pollutants produced in the manufacturing of chemicals, but also among environmental pollutants. Using HPLC combined with electrospray tandem mass spectrometry, we determined that *o*-anisidine is oxidized by rat and rabbit hepatic microsomes to *N*-(2-methoxyphenyl)hydroxylamine, *o*-aminophenol and one additional metabolite, which exact structure has not been identified as yet. In the APCI (atmospheric pressure chemical ionization) mass-spectrum, this metabolite showed the mass signal at *m*/*z* 122.8, corresponding to that of the nitrenium/carbenium ion of *o*-anisidine. *N*-(2-methoxyphenyl) hydroxylamine is either further oxidized to another oxidation product, 2-methoxynitrosobenzene (*o*-nitrosoanisole), or reduced to parental *o*-anisidine, which can be oxidized again to produce *o*-aminophenol. To define the role of microsomal cytochromes P450 (P450) in *o*-anisidine metabolism, we investigated the modulation of this metabolism by specific inducers of these enzymes. The results of the studies suggest that *o*-anisidine is a promiscuity substrate of P450s of rat and rabbit liver; P450s of 1A, 2B, 2E and 3A subfamilies metabolize *o*-anisidine in hepatic microsomes of both studied species. Using purified enzymes (P450s 1A1, 1A2, 2B2, 2B4, 2E1, 2C3, 3A1 and 3A6), reconstituted with NADPH:P450 reductase, the ability of P450s 1A1, 1A2, 2B2, 2B4, 2E1, and 3A6 to metabolize *o*-anisidine was confirmed. In the reconstituted P450 system rabbit P450 2E1 was the most efficient enzyme metabolizing *o*-anisidine.

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REFERENCES

[1] Stiborová M., *et al.* (2005) *Int. J. Cancer* **116:**667–678.

PHENYTOIN ADMINISTRATION DURING PREGNANCY IN RATS – EFFECT ON OFFSPRING

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Phenytoin (PHT), a widely used anticonvulsant, when administered in pregnancy, is thought to cause toxicity in the embryo or foetus via reactive oxygen intermediates [1]. PHT was administered orally from day 2 until day 19 of gestation in the dose of 150 mg/kg. The effect of PHT on teratological, biochemical, structural and behavioural variables in rat foetuses and offspring was evaluated. Embryofoetal toxicity of PHT was manifested by declined body weight gain of the dams and decreased foetal and placental weight, as well as increased incidence of skeletal anomalies. In 20-day-old foetuses, an increase in the activity of the lysosomal enzyme N-acetyl-ß-D-glucosaminidase and a decrease of reduced glutathione (GSH) in the placenta and foetal liver were recorded. In contrast, in one-day-old pups only an increased level of GSH in the liver was found. The biochemical changes were reversible. In myocardial tissue and blood vessels irreversible subcellular alterations were observed. PHT caused neurobehavioural defects – delayed neuromotor development and a deficit in spatial learning. In conclusion, PHT administration resulted in reproductive, biochemical, morphological and behavioural changes in rat offspring.

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REFERENCES

[1] Wells P.G., *et al.* (2005) *Toxicol Appl Pharmacol.* **207**(Suppl 2):354–66.

IMMUNE PARAMETERS IN CHILDREN WITH FOOD ALLERGY

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We examined 250 children with food allergy and healthy controls during years 2006–2008. The aim of our work was to study cellular and molecular mechanisms, which play important role in development of food allergy in atopic children. We determined mRNA expression for selected cytokines IFN-gamma, IL-4, IL-8, IL-10, IL-13 in atopic children with food allergy and healthy children group. Further we studied some parameters of cellular and humoral immunity: screening of specific IgE for food allergens, levels of total IgE, phenotype analysis of leucocytes, proliferative activity of lymphocytes, phagocytic activity of leucocytes and haematological parameters.

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TETRAHYMENA PYRIFORMIS AS A BIOSENSOR ON THE DETERMINATION OF TOXIC ORGANOPHOSPHATES IN BIOLOGICAL MATERIALS

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Over the past decades fresh-water-living ciliated protozoan *Tetrahymena pyriformis* became undoubtedly the species of choice throughout the fields of functional biology, ecology, veterinary and human toxicology, and radiobiology. The purpose of this study was an evaluation of the effect levels of various toxic organophosphate agents for the ciliated protozoan growth axenically in medium consisting of 0.75% PPYS (proteose-peptone, yeast extract with inorganic salts). Effects of dichlorvos, trichlorphon, metathion and VX agent on the viable count and acetylcholinesterase (AchE) activity of *Tetrahymena pyriformis* were investigated in this study. Following parameters were evaluated in each toxic agent: LD_{100}, LD_{50}, and IC_{50} for activity of AChE. LD_{50} values were 9.6, 8.0 mg/l, 47.7 μg/l, and 28.2 ng/l for dichlorvos, trichlorphon, metathion and O-ethyl S-[2-(diisopropylamino)ethyl] ester of methylphosphonothioic acid, respectively; IC_{50} values were 1.2 mg/l, 1.0 mg/l, 745 ng/l, and about 600 pg/l, respectively. From these results we conclude more precise assessment of ecological and biological risks of tested toxic agents.

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HALOGENATED PHENIRAMINES PROVED EFFECTIVE IN MESENTERIC ISCHAEMIA/REPERFUSION

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Pheniramines – a group of H_1 antihistamines, were found to have antiradical activity which may be potentiated by halogen substitution in the antihistamine molecule. Since reactive oxygen species (ROS) are supposed to contribute significantly to tissue damage, the observed antiradical activity of pheniramines may prove beneficial as it could reduce tissue damage. The aim of our work was to study the possible protective effect of pheniramines in the rat model of mesenteric ischaemia/reperfusion (I/R) induced injury along with the role of ROS and to find whether halogenation would influence also the protective effect of pheniramines *in vivo*.

The extent of intestinal damage caused by I/R was recorded, the activity of myeloperoxidase (MPO) was measured, increased free radical production was assessed by the chemiluminescence (CL) of the ileal samples. The number of neutrophils in blood was determined. Pheniramine, chlorpheniramine and brompheniramine 10 mg/kg were administered twice i.p., before superior mesenteric artery occlusion and then before reperfusion.

I/R induced pronounced haemorrhagic intestinal injury. All pheniramines reduced significantly the extent of damage. The protective effect of pheniramines, expected to be influenced by halogenation, was nevertheless almost the same. A reperfusion induced MPO increase was also inhibited by all pheniramines. Comparing to sham operated rats, the CL response of the ileal samples increased after I/R with a further increase caused by pheniramines, probably in association with neutrophil count in blood, which was increased in all groups after reperfusion. Such a potentiation of intracellular CL by pheniramines was observed *in vitro* in human neutrophils, with the potency order pheniramine<chlorpheniramine< brompheniramine.

The results obtained showed a similar protective effect of all pheniramines against mesenteric I/R induced damage. Contrary to the effect of halogenation on pheniramine activity *in vitro*, there were no significant differences in their *in vivo* effects.

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Fe^{2+}-INDUCED CHEMILUMINESCENCE IN TRAUMATIC BRAIN INJURY IN MICE: EFFECT OF PYRIDOINDOLE SME1EC2

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Several metabolic derangements, including influx of calcium ions, tissue lactic acidosis, free radical generation, membrane depolarization by release of excitatory amino acid neurotransmitters, free radical generation and tissue lipid peroxidation (LP) have been implicated in acute head trauma (ATH). Trauma-induced LP is one of the most important factors producing tissue damage in ATH. In the present study the protective effect of the pyridoindole SMe1EC2 on neurological deficit induced by ATH (evaluated by the Sensomotoric Score SC) was investigated. This parameter was correlated with the development of LP, content of endogenous antioxidant glutathione and content of lactate after head trauma. Mice were divided into three groups. Controls received only i.v. bolus injections of saline, ATH animals received i.v. either saline bolus or SMe1EC2 (1.14 mg/kg) within one minute after ATH. The animals were killed 5 hours after ATH. Resistance of neuronal membranes to Fe^{2+}-induced lipid peroxidation detected by chemiluminescence (CL) method was monitored. The CL parameters measured showed brain oxidative damage after ATH. The lag period (T)

in formation of lipohydroperoxides was shortened by 208.3% (from 301 to 144 sec, p<0.01) in the ATH group of mice. In the group of SMe1EC2 treated mice, this CL parameter was close to the controls (p<0.01). The content of glutathione was decreased by 25.9% (from 31.03 to 23.0 nmol/mg prot., p<0.001) after SMe1EC2 treatment, reaching practically control values in ATH mice.

The neurological deficit induced by ATH, as evaluated by Sensomotoric Score, was significantly attenuated by SMe1EC2 administration.

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BRASSININ: A NEW SENSITIZER OF HUMAN LEUKEMIA CELLS HL-60 TO DOXORUBICIN

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Inherited and/or acquired resistance to diverse anticancer drugs is a major obstacle for successful chemotherapy in cancer. This phenomenon, known as multidrug resistance, can be caused by different mechanisms. One such a mechanism is linked to the overexpression of membrane drug efflux pumps, P-glycoprotein or multidrug-resistance associated proteins (MRPs) [1]. We have examined the influence of phytoalexin brassinin (3-(S-methyldithiocarbamoyl) aminomethyl indole) on the activity of MRP1 pump and its ability for reverse drug resistance. We assessed the ability of this phytoalexin to function as a chemosensitizers using cytotoxicity assays and calcein efflux assays in human leukemia cell line HL-60 and resistant subline HL-60/ADR overexpressing MRP1. In flow cytometry experiments, accumulation of calcein (a fluorescent substrate of MRP1) was significantly increased at BRA concentrations 100 μM. At a sub-toxic concentration, brassinin (<75 μM) was able to sensitize both HL-60 and HL-60/ADR against doxorubicin and apoptosis of cells has been confirmed. 50% inhibition of cell proliferation was achieved when HL-60/ADR cells were co-treated with 45 nM doxorubicin and 75 μM brassinin.

The inhibition of MRP1 pump by brassinin was evaluated for the first time and this natural substance has been successfully used as sensitizer of leukemia cells for doxorubicin. Results of our *in vitro* study indicate that indolic phytoalexins can bring benefit to anticancer therapy.

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REFERENCES

[1] Borst P. *et al.* (2000) *J. Natl Cancer Inst* **16:** 1295–302.

BIOLOGICALLY ACTIVE MOLECULES IN SALIVARY GLANDS OF *LUCILIA SERICATA* AND HUMAN NEUTROPHIL ACTIVATION

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Maggots of some Dipteran species are used to disinfect chronic wounds by mechanically removing bacteria and by releasing compounds that kill microorganisms. The aim of our study was to analyze effects of extracts from the salivary glands of *Lucilia sericata* on isolated human neutrophils, as well as to test their antimicrobial activity.

Neutrophils are an essential part of the nonspecific immune system, also known as innate immunity, which keep organisms healthy in an environment of potentially pathogenic microorganisms. The system includes both passive and active mechanisms that are readily available during the early phases of pathogen – host cell molecular interactions, host response and inflammation. To study the effect of extracts from salivary glands of *Lucilia sericata* on human neutrophils (from peripheral blood of healthy donors) *in vitro*, we used a receptor operating stimulus, opsonized zymosan (OZ), to evoke respiratory burst, measured as superoxide generation, and myeloperoxidase (MPO) release as a marker of degranulation. To differentiate the membrane effect of the extract from its direct effect on MPO activity, we compared its effect on cell free system: crude extract from neutrophils, horse radish peroxidase and purified commercial human MPO. The extract had no effect on resting neutrophils but decreased OZ stimulated superoxide generation and MPO release.

The antimicrobial activity of the extract from salivary glands was determined by plate-growth inhibition assay. As model microorganisms for these tests, gram-positive bacteria *Bacillus subtilis, Micrococcus luteus, Sarcina lutea* and gram-negative bacteria *Escherichia coli* and *Seratia marcescens* were used. Moreover, the effect of HPLC purified fractions on some clinically important microorganisms, (*Pseudomonas aeruginosa, Staphylococcus aureus, Klepsiella* sp., *Bacteroides* sp.) were tested for their antimicrobial activity. The extracts significantly inhibited the growth of gram-positive bacteria, yet not of the gram-negative bacteria *E.coli.*

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ALDOSE REDUCTASE INHIBITION ABOLISHES GLUCOSE-INDUCED ENDOTHELIAL DYSFUNCTION

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Increased glucose utilization by aldose reductase, a rate-limiting enzyme of the polyol pathway, has been

implicated in the pathogenesis of diabetic vascular complications. In this process, several biochemical mechanisms are involved, including depletion of reduced cofactors necessary for action of antioxidant enzymes or endothelial NO synthase. In this study, the effect of a novel aldose reductase inhibitor JMC2004 on hyperglycemia-induced endothelial dysfunction was studied.

Bovine aortic endothelial cells (BAEC) were treated with glucose (30mM), JMC2004 (0.01mM), or glucose + JMC2004 for 24 h. Then the cells were stimulated with 0,001mg/ml of calcium ionophore A23187 and NO production was measured electrochemically using porphyrine-coated carbon NO electrode. After the measurement, cell supernatants were harvested and nitrite concentrations were evaluated using Griess reaction. Further, peroxyl and hydroxyl radical-scavenging activity of JMC2004 was measured with luminol–enhanced chemiluminescence. Superoxide scavenging was measured colorimetrically using XTT.

24h incubation of the cells with 30mM glucose strongly diminished calcium ionophore-induced response. Concomitant treatment with JMC2004 restored NO production by 50%. This effect was probably antioxidant-independent, since JMC2004 did not exert any scavenging activity towards any of tested radicals. In conclusion, aldose reductase inhibition with JMC2004 was able to abolish hyperglycemia-induced endothelial dysfunction in bovine aortic endothelial cells.

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ANTIOXIDATIVE EFFECT OF DERIVATIVES OF STILBENE. COMPARISON OF TRANS-RESVERATROL, PINOSYLVIN AND PTEROSTILBENE

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Plants are a rich source of active substances, which are used in therapy of a wide range of diseases related to oxidative stress, e.g. rheumatoid arthritis. The main well of pathologically working reactive oxygen species (ROS) are activated neutrophils. Taxonomically different plant species may include chemically related derivatives with similar action. Aim: There are few papers on some antioxidant effects of vegetal substances from the stilbenoid group. We compared the effect of *trans*-resveratrol, which is well-known by its antioxidative activity, with the effect of pinosylvin and pterostilbene.

We used luminol-enhanced chemiluminescence (CL) to study the antioxidative action. The effect was observed in whole blood and in isolated neutrophils. The concentrations of substances tested were 0.01–100 μM. Due to the different abilities of luminol and isoluminol to pass through the cell membrane, we studied the effect of the substances tested on intracellular and extracellular ROS. To stimulate the production of ROS we used phorbol-myristate-acetate (PMA), which activates neutrophils *via* protein kinase C.

Resveratrol, pinosylvin and pterostilbene inhibited significantly the CL of whole blood in a dose-dependent manner. All three substances tested also significantly inhibited the extra- and intracellular CL of isolated neutrophils. The inhibitory effect of individual substances was different in whole blood, extra–, and intracellular milieu of neutrophils.

The presence of different functional groups in the molecules of stilbenoids influence their antioxidative effect. Modification of these functional groups may result in derivatives which could significantly inhibit toxic activities of ROS.

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IN VITRO TOXICITY OF MOULD GROWTHS ON DIFFERENT BUILDING MATERIALS

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In vitro toxicity of complex chloroform-extractable endo- and exometabolites of representative indoor, and related outdoor, fungal isolates from 80 dwellings in Slovakia – mouldy nad control ones – has been evaluated by a bioassay with 1-day-old chicks´ tracheal organ cultures. Micromycetes, mostly *Aspergillus versicolor* (able to synthetize a mycotoxin sterigmatocystin detected by LC/MS-MS), *A. flavus* (non-aflatoxigenic according to TLC), other aspergilli, penicillia (potential sources of wide mycotoxins´ spectrum based on TLC analysis), produced secondary metabolites that ceased ciliary beating in tracheal epithelium in the organ cultures already in 24 hrs of the activity, i. e. in the sense of the method used, they belong to strong toxicants. Nineteen of 55 mould isolates tested so far produced also extrolites without toxic effects detectable by the method. It has been proven that toxin production in fungi depends not only onto the species but may vary between every single isolates as well. The most important outcome of the study is that microscopic filamentous fungi present in the dwelling indoor environment under Slovak (Central European) building/housing conditions might produce compounds even with a strong potential to damage upper airways of occupants, while children remain the most vulnerable population.

The study was a part of the project APVT-21-052102.

ACETYLCHOLINESTERASE BASED BIOSENSORS FOR NERVE AGENTS ASSAY

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Man-made organophosphates and organophosphonates represent a harmful group of compounds employed

in several ways especially as nerve agents and pesticides. The toxicity effect towards humans and animals is caused by the inhibition of cholinesterase activity in living organism. Detection devices for organophosphates could be based on different principles; however, the one based on recognition capabilities of cholinesterase seems to be quite approachable.

Presented work is concerning to performance biosensors and simple strip sensors for nerve agents and less toxic insecticides assay. Capability of developed method was tested on sarin, paraoxon-ethyl, paraoxon-methyl, and methamidophos. Electrochemical strips containing platinum working, Ag/AgCl reference and platinum auxiliary electrodes were employed for biosensor construction commonly with human recombinant acetylcholinesterase. Digestion of acetylthiocholine by enzyme and consequent oxidation of thiocholine on electrode surface at applied voltage 450 mV was used for enzyme activity monitoring. Two variants of cholinesterase application for amperometric biosensor construction were applied. Firstly, cholinesterase was in the homogenous phase with acetylthiocholine commonly placed into reaction microchamber. Secondary, cholinesterase chemically bound on platinum surface was performed.

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MECHANISM OF ELLIPTICINE ACTION ON NEUROBLASTOMA CELLS

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Ellipticines are plant alkaloids with an antineoplastic activity. The mode of action is based mainly on DNA intercalation, inhibition of topoisomerase II and formation of covalent DNA adducts mediated by cytochromes P450 and peroxidases. Such ellipticine-DNA-adducts are formed *in vitro*, in human breast adenocarcinoma MCF-7, leukemia HL-60 and CCRF-CEM cell lines and *in vivo* in rats and mice exposed to ellipticine. Here, the cytotoxicity of ellipticine to human neuroblastoma cell lines IMR-32, UKF-NB-3, UKF-NB-4 and UKF-NB-4^{elli} (the cell line possessing a partial resistance to ellipticine) was investigated. Furthermore, the effect of hypoxic conditions on the ellipticine cytotoxicity to these cells was also examined.

The toxicity of ellipticine to neuroblastoma cell lines cultivated under the standard conditions was higher than that to the cell lines grown under the entire lack of oxygen (1%). Using the ^{32}P-postlabeling assay, ellipticine-DNA adducts were detected in all neuroblastoma cells. A pattern of DNA adducts showed formation of two major deoxyguanosine adducts, identical to adducts derived from 13-hydroxy- or 12-hydroxyellipticine, metabolites formed from ellipticine by CYP enzymes of 3A and 1A subfamilies or from metabolites generated by peroxidases. Total levels of DNA adducts generated by 10 μM ellipticine in cell lines used in the study differ significantly, being the highest in IMR-32 cells (26 adducts per 10^7 nucleotides), while 8 and 17 adducts per 10^7 nucleotides were formed in UKF-NB-3 and UKF-NB-4 cells, respectively. The cell cultivation under the hypoxic conditions mediated a decrease in toxicity of ellipticine to these cells. Such a lower sensitivity to ellipticine correlates with a decrease in the formation of ellipticine-derived DNA adduct. The results suggest that the formation of covalent DNA adducts by ellipticine is one of the most important mechanisms of ellipticine action to neuroblastoma cells.

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EFFECT OF SESAME OIL BASED PHYTOTHERAPY ON OXIDATIVE STRESS PRESENT IN ADJUVANT ARTHRITIS

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Oxidative stress (OS), as a result of increased production of reactive oxygen and nitrogen species (ROS/RNS), is a hallmark of chronic inflammatory diseases including rheumatoid arthritis (RA). Animal studies document increased levels of ROS/RNS in joints and plasma of animals with adjuvant arthritis (AA). Experimental studies have documented positive correlations of markers of OS and disease activity. The aim of this study was to evaluate the effects of sesame oil (SEO) and three plant extracts, together dissolved in SEO, on OS and the development of AA. The SEO and plant extracts from *Boswellia serrata (BO)*, *Zingiber officinale (Zg)* and *Arctostaphylos uva ursi (AUU)* were chosen because of their antioxidant and/or anti-inflammatory effects. The experiments included healthy Lewis rats, arthritic animals without any drug administration, arthritic animals with administration of three plant extracts (BO 50 mg/kg b.w., Zg 25 mg/kg b.w., AUU 25 mg/kg b.w.) together dissolved in emulsion of SEO and water, and arthritic animals with SEO administration in the same volume as the mixture with plant extracts. The treatment involved daily oral administration of the substances from day 1, i.e. the day of immunization *(Mycobacterium butyricum* suspended in incomplete Freund´s adjuvant) to the end of the experiment – day 28.

The arthritic parameter (hind paw volume) was decreased by the administration of SEO and three plant extracts only moderately. Markers of oxidative stress (TBARS, protein carbonyls and γ-glutamyltransferase)

were reduced by SEO and the three plant extracts. A significant reduction of plasmatic protein carbonyls, plasmatic levels of TBARS and the activity of γ-glutamyltransferase in spleen and joint homogenates was observed in arthritic animals treated with the three plant extracts. SEO significantly decreased only the plasmatic levels of TBARS. In conclusion, an effective antioxidant-based drug that attenuates OS could have beneficial therapeutic effects in RA patients.

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HIGH-MOLAR-MASS HYALURONAN BEHAVIOR DURING TESTING ITS ANTIOXIDANT PROPERTIES IN ORGANIC AND AQUEOUS MEDIA: EFFECTS OF THE PRESENCE OF MN(II) IONS

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This study compares the antioxidant activity of high-molar-mass hyaluronan (HA) using standardized methods applying 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals and 2,2'-azino-di-[3-ethylbenzthiazoline sulphonate] (ABTS) radical cations as oxidants. Additionally spin-trapping technique combined with electron paramagnetic resonance was used to evaluate the ability of HA to scavenge reactive radicals. The thermal decomposition of $K_2S_2O_8$ in pure H_2O or in a H_2O/dimethylsulphoxide (DMSO) mixture at 333 K was used as a source of reactive paramagnetic species. We found that HA does not exhibit antioxidant activity if DPPH radicals or $ABTS^{\bullet+}$ radical cations are used as the oxidant, but that hyaluronan is an effective radical scavenger at low concentrations if the oxidation reactions are initiated by the decomposition of $K_2S_2O_8$. At higher HA concentrations more complex behavior and prooxidant HA action was indicated. The influence of Mn(II) ions on the reaction mechanisms of radical generation and termination in the $K_2S_2O_8/H_2O$/DMSO system in the presence of HA was studied in detail.

PREVALENCE OF ALLERGIC DISEASES IN 3-YEAR-OLD CHILDREN IN BRATISLAVA – COMPARISON BETWEEN 1999 AND 2005

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Environmental factors play an important role in development and manifestation of allergic diseases. Children represent a subpopulation especially vulnerable to the exposure to environmental pollution.

Aim of the study was to compare the prevalence of allergic diseases in 3-year-old children between 2 time periods in Bratislava.

Two groups of 3-year-old children, born in Bratislava, were examined for allergic diseases in 2 different time periods. The first group was examined in 1999 (n=62), the second one in 2005 (n=70). Clinical examination for development of allergic diseases (ADs) including atopic eczema (AE), asthma respiratory symptoms (ARS), hay fever (RA) and food allergy (FA) was performed by the same allergist and under the same conditions.

The prevalence of ADs in children differed between 1999 and 2005. In general, prevalence of ADs (54.8% vs. 24.1%,), AE (29.0% vs. 14.3%,), ARS (16.1% vs. 4.3%), RA (6.5% vs. 1.4%), and FA (16% vs. 5.7%) was higher in children born in 1999, if compared to children born in 2005, respectively. Significant differences were found only for ADs ($p<0.001$) and ARS ($p=0.04$).

The decreasing prevalence of ADs in children might be explained by reduction of urban air pollution within the region between the two time periods. Improvement of air quality parameters in Bratislava was supported by the work of Mišík et al. (2007), who found decrease in genotoxic effects of ambient air pollution in Bratislava comparing periods 1997–2000 and 2003–2006.

Significant difference in ADs prevalence were found in 3-year-old children between 1999 and 2005 that may be the result of changes in air pollution, e.g. introduction of new and cleaner industrial technologies.

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MELATONIN AMELIORATES NITRO-OXIDATIVE DAMAGE AT THE MOLECULAR LEVEL

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Melatonin is an uncommonly effective direct free radical scavenger and indirect antioxidant. Within the last decade a plethora of studies have documented melatonin's ability to neutralize both reactive oxygen (ROS) and reactive nitrogen secies (RNS). Melatonin detoxifies the superoxide anion radical, hydrogen peroxide, nitric oxide, peroxynitrite anion and hypochlorous acid. Moreover, when melatonin functions as a direct free radical scavenger it produces by-products, for example, cyclic 3-hydroxymelatonin, N1-acetyl-N2-formyl-5-methoxykynuramine, N1-acetyl-5-methoxykynuramine, etc., all of which are also excellent scavengers of ROS/RNS. This series of reactions is referred to as melatonin's antioxidative cascade and permits a single molecule of melatonin to scavenge possibly up to ten toxic reactants. Additionally, melatonin stimulates the gene expression and activities of several antioxidative enzymes including the superoxide dismutases, glutathione peroxidase and glutathione peroxidase. Also, it promotes the synthesis of another important intracellular antioxidant, glutathione, by activating

gamma-glutamyl cysteine synthase. Finally, the melatonin metabolite, N1-acetyl-5-methoxykynuramine, inhibits the proxidative enzyme, nitric oxide synthase. Via these multiple actions, melatonin has proven highly effective in reducing the quantity of intracellular molecular debris normally left in the wake of free radicals and related reactants.. Via these means, melatonin is highly effective in combatting molecular damage due to ionizing radiation and other toxic agents that can be environmentally-dispersed. Many drugs in common usage also are toxic because they generate free radicals at the mitochondrial level and in the cytosol. This damage is reduced when melatonin is also present. Finally, in experimental studies, melatonin has been found to limit heavy metal toxicity including that from aluminium, arsenic, cadmium, chromium, copper, lead, mercury, nickel and many others. Melatonin is virtually withourt toxicity at any dose and can be administerd via any route making it highly useful under a variety of conditions.

TOXICOLOGICAL TESTING RELATED TO REACH

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On 18 December 2006 the Council of Ministers adopted a new EU regulatory framework for **R**egistration, **E**valuation, **A**uthorisation and Restriction of **Ch**emicals (REACH). The main aims of REACH are to improve the protection of human health and the environment from the risks that can be posed by chemicals, the promotion of alternative test methods, the free circulation of substances on the internal market and enhancing competitiveness and innovation.

REACH makes industry responsible for assessing and managing the risks posed by chemicals and providing appropriate safety information to their users.

Since it came into force in June 2007 REACH requires that all chemicals manufactured in or imported into the European Union in volume of one **tonne** or more each year have to be tested for health and safety and registered with a new central European authority.

hameln rds a.s. Modra testing methods related to the REACH regulation are presented.

DOSE-RESPONSE EFFECTS OF NATURAL AND SYNTHETIC ESTROGENS ON PROGESTERONE PRODUCTION IN PORCINE OVARIAN GRANULOSA CELLS

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Despite the structural diversity of exogenous natural and synthetic estrogens, all of them have the capacity at some concentrations to bind to estrogen receptors in target cells of the body and initiate or inhibit estrogen-like actions. Estrogen mimics have the potential to alter growth, development, and function of estrogen target tissues. This study was designed to compare the *in vitro* effects of natural estrogens (estradiol, genistein, coumestrol) and different synthetic estrogens (diethylstilbestrol, bisphenol A (BPA), di(2-ethylhexyl) phthalate (DEHP)) on basal and gonadotropin-stimulated progesterone production in porcine ovarian granulosa cells.

Granulosa cells isolated from porcine ovarian follicles (4–6 mm) were incubated with the tested compounds (10^{-10}–10^{-4} M) in the presence or absence of follicle-stimulating hormone (FSH) (1 μg/ml) for 72 h. At the end of the incubation, progesterone levels produced by granulosa cells were measured in the culture media by radioimmunoassay.

All tested agents at 10^{-5} and 10^{-4} M concentrations, except DEHP, exerted an inhibitory effect (decrease about 50 to 80%) on basal as well as FSH-induced progesterone synthesis by granulosa cells. BPA at 10^{-4} M also decreased progesterone production by granulosa cells after stimulation with luteinizing hormone (1 μg/ml) or forskolin (10 μM), respectively. The decrease was associated with the inhibition of P450scc activity as well as with the decrease in cAMP levels. On the contrary, DEHP induced stimulation (about 15 to 60%) of basal progesterone synthesis in a concentration-dependent manner and enhanced FSH-induced progesterone levels in cell culture media at all tested concentrations.

There is growing evidence that possible toxicological/benificial effects of the xenoestrogens may not necessarily be mediated through competitive binding with estrogen receptor. We suppose that intracellular enzymes involved in steroidogenesis might be implicated in the action of xenoestrogens.

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SUDAN I IS OXIDIZED BY PEROXIDASES TO SPECIES FORMING (DEOXY)GUANOSINE ADDUCTS IN DNA

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1-Phenylazo-2-hydroxynaphthalen (Sudan I) is a liver and urinary bladder carcinogen in mammals. We have found that *in vitro* Sudan I is oxidized by cytochromes P450 (CYP), predominantly by CYP1A1 and peroxidases. Peroxidase systems oxidize Sudan I to one- electron oxidation products, which are able to covalently modify DNA, RNA and proteins. These covalent adducts differ from those formed from Sudan I activated by cytochromes P450. The carcinogen oxidized by peroxidases form DNA adducts, which are similar to those formed in rat urinary bladder *in vivo*, one of the target tissues for Sudan I carcinogenicity, which is rich in these enzymes. Therefore, the structural characterization of these adducts is the aim of our present work.

Sudan I is oxidized by peroxidase to eight metabolites. In the presence of deoxyaguanosine (guanosine),

Sudan I-dG/G adducts are also formed during the reaction. Sudan I metabolites and adducts formed from Sudan I with guanosine and/or deoxyguanosine were isolated utilizing combination of extraction, TLC and HPLC methods and characterized using mass and NMR spetroscopy. Two of the major Sudan I metabolites (assigned as metabolites M_1 and M_2) were structurally characterized. One of these Sudan I metabolite (M_2) is the primary product formed during oxidation of Sudan I to radicals, which generate Sudan I dimer. The second metabolite (M_1) is the product of secondary, enzyme independent reactions, being formed from the Sudan I dimer that lost the benzenediazonium moiety. Two major adducts formed during the peroxidase oxidation of Sudan I with (deoxy)guanosine were separated by TLC and/or HPLC and characterized by UV/VIS and mass spectroscopy as well as by NMR. The structural characterization of these adducts indicate that one of the adducts is formed by the binding of metabolite M_1 to the exocyclic NH_2 group of a guanine residue of (deoxy) guanosine. The second adduct is the compound, in which radical localized in position 4 of the naphthalene ring of the Sudan I molecule is bound the same position of a guanine residue moiety, the exocyclic NH_2 group. The results are discussed from the point of view of the Sudan I carcinogenicity to the urinary bladder.

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PCR DETECTION OF GLUTEN CONTAINING CEREALS IN BAKERY PRODUCTS

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Gluten enteropathy (coeliac disease) is a disease caused by an inappropriate immune response to dietary wheat, barley or rye gluten. Patients suffering of this disease have to exclude gluten-containing cereals from their diet. For these consumers, a special category of food products designated "gluten-free" is produced [1]. Contamination of these food products by gluten-containing cereals may occure. Polymerase chain reaction (PCR) represents an effective alternative for the detection of wheat or other gluten-containing cereals by immunochemical methods. PCR has a potential for sensitive screening of gluten-containing cereals in food products.

Using DNA purified from flour, an intrinsic detection limit of 42 ± 12 pg was determined, which corresponds to 1–9 gene copies. The PCR was applied to 26 high bread wheat cultivars used in Europe. All cultivars were positively detected which means that the inclusivity of the primers was 100%. The primers used were specific for wheat, barley and rye. To obtain data on the exclusivity of the primers, non-gluten-containing crops were tested, too. All six crops produced negative PCR results, so the exclusivity was 100%.

The detection limit of 0.1% (w/w) was determined that confirms the suitability of method for the analysis of gluten-free foods, as it is equivalent to the limit required by food standards. The applicability of the method was tested on 17 real products. Two flours and one biscuit declared as gluten-free were positive for gluten containing cereals.

Application of the method to these samples proved that it was suitable for routine use, it was relatively straightforward and could be completed in one working day.

This study has been supported by the Research project of the Ministry of Agriculture "Development of progressive methods and practices for continuous quality improvement in the process of food production and monitoring".

REFERENCES

[1] Joint FAO/WHO food standards programme CodexAlimentarius Commission (2002). Alinorm, 03/26.

INTERACTION OF SELECTED INHIBITORS OF CYCLIN DEPENDENT KINASES WITH HUMAN LIVER MICROSOMAL CYTOCHROMES P450

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Inhibitors of cyclin dependent kinases based on N^6 substituted benzylaminopurine structure as olomoucine II and roscovitine have attracted attention due to their ability to cause the blocking of the animal cell cycle. Hence, these compounds act as potential antineoplastic drugs [1]. Liver microsomal cytochromes P450 (CYPs) belongs to the enzymes of the first phase of metabolism of xenobiotics participating in metabolism of various drugs. Interactions between CYPs and drugs can lead not only to drug metabolism but also to unwanted effects such as enzyme inhibition or induction. These latter effects are responsible for so called drug-drug interactions [2]. A little is known about the interaction of olomoucine II and roscovitine with human liver microsomal CYPs. Therefore, a systematic study examining the potential inhibition of activities of important drug-metabolising CYPs (CYP1A2, 2A6, 2B6, 2D6, 2C9, 2C8, 2C19, 2E1 and CYP3A4) by olomoucine II and roscovitine has been performed. Potential participation of some CYPs in biotransformation of the respective substances was also studied. Olomoucine II as well as roscovitine inhibited CYP1A2 and CYP2C9 activities, moreover olomoucine II influenced to certain extent also the CYP3A4 activity. Roscovitine exhibits rather specific inhibition of the CYP1A2 activity with Ki of 15 μM and of the CYP2C9 activity with Ki of 40 μM. Olomoucine II causes an inhibition of the CYP1A2 activity with Ki value of 35 μM. The mixed mechanism of inhibition takes place in all cases mentioned. Olomoucine II is probably

metabolised by several CYPs with CYP3A4 being the most important primarily. The LC/MS identification of the purported olomoucine II metabolite is in the stage of development.

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REFERENCES

[1] Hajduch M, *et al.* (1997) *Anticancer Drugs* **8:**1007–1013.
[2] Bachmann KA, *et al.* (2004) *Drug interactions handbook*. 2nd ed. Hudson, OH: Lexi-Comp.

CYTOCHROME P450 PARTICIPATES IN DETOXICATION OF CARCINOGENIC ARISTOLOCHIC ACID

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Aristolochic acid (AA), the plant extract of *Aristolochia* species, was recently proven to be associated with the development of aristolochic acid nephropathy (AAN), which is characterized by chronic renal failure, tubulointerstitial fibrosis and urothelial cancer. AA may also cause a similar type of kidney fibrosis with malignant transformation of the urothelium, the Balkan endemic nephropathy (BEN). One of the common features of AAN and BEN is that not all individuals exposed to AA suffer from nephropathy and tumor development. We have suggested earlier that one cause for these different responses may be individual differences in the activities of the enzymes catalyzing the detoxication and/or activation of AA. Namely, the genetic variations of enzymes metabolizing xenobiotics appear to be important determinants of their toxic effects. Therefore, understanding which enzymes are involved in AA activation and/or detoxication is important in the assessment of an individual's susceptibility to this carcinogen.

While the enzymes activating the major component of AA, AAI, *in vitro* have already been identified [1], those participating in AAI detoxication both *in vitro* and *in vivo* have not yet been investigated in details. In this study we investigated the detoxication of AAI, namely its demethylation to aristolochic acid Ia (AAIa). We found that human and rat hepatic microsomes are capable of oxidizing AAI to AAIa. HPLC was used to separate this metabolite from AAI, being eluted at retention time of 28.5 min as an individual product peak. Cytochrome P450 (CYP) enzymes were identified to be responsible for formation of AAIa. To define the role of microsomal CYP enzymes in AAI oxidation, we investigated the modulation of this reaction by specific inducers and selective inhibitors of these enzymes. Furthermore, we also used microsomes of Baculovirus transfected insect cells containing recombinant human CYPs and NADPH:CYP reductase (Supersomes™). The results of the studies suggest that AAI is a promiscuity substrate of CYP enzymes, being oxidized by CYPs of several subfamilies. However, the relative contribution of CYP enzymes detoxicating AAI *in vivo* remains still to be resolved.

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REFERENCES

[1] Stiborová M., *et al.* (2008) *Mutat. Res.* **658:**55–67.

ECOTOXICOLOGICAL EVALUATION OF DANGEROUS SUBSTANCES FOR ESTABLISHMENT OF THE ENVIRONMENTAL QUALITY STANDARDS (EQSS) FOR WATER

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Because of rivers are reported to be contaminated by either partially treated or untreated discharges from various sources of pollutants, there is a need to evaluate the effluents and develop the strategies to reduce and prevent the water contaminations. One of the possibilities is to recognize and to characterize the substances dangerous to water. Ecotoxicological tests represent an important methodological approach to the identification, characterization and assessment of dangerous substances. The aim of the study is ecotoxicological evaluation which helps to develop the Environmental Quality Standards (EQSs) for some of priority substances and dangerous substances, where no data on dose-response are available in the literature. We evaluated six dangerous substances (Benzensulfonamide, Benzothiazole, 4-methy-2,6-diterbuthylphenol, Bisphenol A, Dibutylphthalate, Phenanthrene) in various ecotoxicological tests within the framework of the project PHARE-TWINNING SK 05/IB/EN/01 "Establishment of the Environmental Quality Standards (EQSs) for water and strengthening of regional and district environmental offices for implementation of water controls and monitoring".

Final results are reported in table 1 and table 2. Results of ecotoxicological studies were used for the establishment of EQS for priority pesticides relevant in the aquatic environment in Slovak Republic.

Table 1. ACUTE TOXICITY

Name of the test substance	DIT		FAT		AGI	
	EC_{50} (mg/l)	NOEC (mg/l)	EC_{50} (mg/l)	NOEC (mg/l)	EC_{50} (mg/l)	NOEC (mg/l)
Benzothiazole	26.98	20.00	*	*	149.79	100.00
Butylhydroxytoluene	*	*	>1.00	1.00	*	*
Bisphenol A	8.54	5.00	*	*	21.74	7.00
Dibutylphthalate	*	*	*	*	8.23	5.00
Phenanthrene	*	*	*	*	4.06	1.50
Benzensulfonamide	449.90	300.00	274.33	100.00	184.70	100.00

* – not tested
DIT – Daphnia immobilization test, OECD 202, C.2, STN EN ISO 6341
FAT – Fish acute toxicity test, OECD 203, C.1, STN EN ISO 7346-1
AGI – Algal growth inhibition test OECD 201, C.3, STN EN ISO 8692

Table 2. LONG TERM TOXICITY

Name of the test substance	FPT		DRT	
	EC_{50} (mg/l)	NOEC (mg/l)	EC_{50} (mg/l)	NOEC (mg/l)
Benzothiazole	*	*	83.82	<0.10
Benzensulfonamide	325.41	200.00	37.61	<1.00
Butylhydroxytoluene	>10.00	10.00	*	*

* – not tested
FPT – Fish prolonged toxicity, OECD 204
DRT – Daphnia reproduction toxicity, OECD 211, C.20, STN ISO 10706

OXIDATIVE STRESS INVOLVEMENT IN FUNCTIONAL DISTURBANCES DURING ADJUVANT ARTHRITIS DEVELOPMENT IN LEWIS RATS

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Adjuvant arthritis is a model of chronic inflammation that exhibits several pathological changes similar to those occurring in rheumatoid arthritis, an autoimmune disease in humans. Due to inflammation processes, the levels of nitric oxide (NO) produced by iNOS are elevated in adjuvant arthritis. However, in vessels, endothelial dysfunction is considered to represent reduced bioavailability of NO, which is a major endothelium-dependent vasodilator. Decreased NO concentration in the vessels then probably reflects its increased interaction with overproduced oxygen free radicals rather than reduced production.

The goal was to study the correlation between vascular functional disturbances and the development of adjuvant arthritis represented by increase of hind paw volume and changes of selected biochemical parameters as a result of oxidative tissue injury.

Adjuvant arthritis was induced by *Mycobacterium butyricum* in Freund's adjuvans. The development of hind paw swelling and endothelium-dependent relaxation of the aorta, identified as the response to acetylcholine *in vitro*, were determined on days 14 and 28 following adjuvant administration. At the same time, concentrations of NO and glutathione in the kidney and aorta were assessed.

Maximal arthritic changes in the hind paw were found on day 28 after adjuvant administration. Similarly, significant decrease of aortal endothelium-dependent relaxation was observed on day 28.The levels of glutathione in the kidney and aorta were decreased on day 14 and they returned to control levels on day 28. This probably reflects reduced antioxidant defence of the organism in the first phase of the arthritic process, followed by the onset of adaptation mechanisms. We observed the same situation in the case of NO concentrations in the aorta and the kidney – initially a decrease and then return to the control values.

From our results we conclude that changes in NO and glutathione levels preceded functional disturbances in the hind paw and aortic endothelium in the course of adjuvant arthritis.

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THE EFFECT OF OXIME REACTIVATOR HI-6 ON THE CHOLINERGIC SYSTEM OF THE RAT BLADDER

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The current treatment of poisoning with organophosphorus compounds (sarin, tabun, soman, paraoxon, parathion) is provided by oxime reactivators and anticholinergic drugs since the effect of these poisons is caused by inhibition of acetylcholinesterase (AChE, EC 3.1.1.7) and subsequent cumulation of acetylcholine (ACh) at the synaptic clefts. Since no versatile cure is known, other mechanisms should be study. HI-6 seems to be the most efficacious oxime for the antidotal treatment because of the highest reactivating and therapeutic efficacy. This high therapeutic potency, besides its reactivating potency, may be also due to other antidotal mechanisms based on direct antimuscarinic action. HI-6 has not only reactivating but also an inhibitory potency on AChE.

In our work, we have investigated an influence of HI-6 on the AChE and on the muscarinic receptors. The study was conducted on rat bladder using *in vitro* test (tissue bath; methacholine as muscarinic agonist). We assume that the response that has been seen can be attributed to the inhibition of the AChE at the lower concentration and to a predominant allosteric inhibition of muscarinic receptor at higher concentration of HI-6.

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MODULATION OF CELL SIGNALING BY CHEMICAL CARCINOGENS

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Cancer is considered to be the end result of a multistage process in which a large number of endogenous and exogenous factors interact, to disrupt normal cell growth and division. The process of chemical carcinogenesis has been divided into two major stages, initiation and promotion. Initiation describes the process whereby a chemical compound damages the DNA of the cell. This process can be modulated by factors that change the efficiency of DNA repair or modify biotransformation of chemicals that require metabolic activation before they can covalently bind into DNA. Tumor promotion is

an essential process in multistage cancer development providing the conditions for clonal expansion of initiated/preneoplastic cells. A continuous disturbance of cellular signal transduction results in stimulation of cell proliferation, cell motility or inhibition of apoptosis.

Mitogen-activated protein kinases (MAPKs) are a conserved family of serine/threonine protein kinases involved in various cellular functions such as proliferation, differentiation, migration and apoptosis. MAPK signaling cascades can be activated by a wide range of different stimuli acting through cell surface receptor families or external/environmental stresses. At least four members of the MAPK family have been identified – extracellular-signal-regulated kinase 1/2 (ERK1/2), c-Jun-amino-terminal kinase (JNK), p38 and ERK5.

The goal of this study was to evaluate the capacity of several tissue specific carcinogens to activate the MAPK signaling pathway in the rat epithelial `stem like´ liver WB-F344 cells. Two strict hepatocarcinogens, 5,9-dimethyldibenzo[*c,g*]carbazole (diMeDBC) and aflatoxin B1 (AFB1), two specific sarcomagens, *N*-methyldibenzo[*c,g*]carbazole (MeDBC) and benzo[a] pyrene (BaP), and *7H*-dibenzo[*c,g*]carbazole (DBC) with both hepatocarcinogenic and sarcomagenic activities were used. Up-regulation of MAPKs due to cell exposure to carcinogens was assessed by western blot analysis using specific antibodies at several time intervals (30, 60, 90 and 120 min) during cell treatment. A noticeable up-regulation of ERK1/2 activity was determined in AFB1-treated cells at all time intervals. BaP induced time-dependent increase of pERK1/2; the highest pERK1/2 level was detected at 120 min. sampling time. A variable up-regulation of ERK1/2 was determined in DBC-, MeDBC-, and diMeDBC-treated cells as well.

Our data suggested that modulation of MAPK pathway might contribute to chemical carcinogenesis. Further experiments are necessary to elucidate this phenomenon.

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ANTIOXIDANT STATUS IN BLOOD SAMPLES OF AIRCREWS OCCUPATIONALLY EXPOSED TO RADIATION AND STRESS FACTORS

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Ionizing radiation triggers the formation of free radicals, which interact among themselves and with critical biological targets causing oxidative damage of biomolecules and consequently may be responsible for the genomic instability. The biological activity of these highly reactive compounds is controlled *in vivo* by several defence mechanisms.

An antioxidant status indicates the degree of endogenous protection against radiation-induced oxidative damage. Imbalance between oxidative stress and antioxidant defence mechanism is associated with a number of diseases, including cancer.

In a small biomonitoring trial of aircrews we analysed activities of antioxidant enzymes glutathione peroxidase (GPX), glutathione S-transferase (GST), superoxide dismutase (SOD) and catalase (CAT) in erythrocytes of investigated subjects. A total antioxidant capacity (FRAP), ceruloplasmin oxidase activity (CPL) and lipid peroxidation (malondialdehyde) were measured in plasma.

A total of 98 subjects were recruited from two airlines, 58 pilots (average age 37 years), and a reference group of 40 ground crews without a history of frequent airline travel (average age 32 years). All study participants signed an informed consent form and the study was approved by the Ethical Committee of the Slovak Medical University in Bratislava.

Preliminary results show a statistically significant decrease in levels of SOD in group of pilots compared with reference group (p=0.005), although no difference was find in malondialdehyde levels.

There were no significant differences found in other antioxidative enzymes: CAT, GPX and GST, and CPL activity between two monitored groups.

After study is completed associations with exposure to radiation as well as possible interactions with other biomarkers and antioxidant parameters will be evaluated.

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ACTIVATION AND DETOXICATION OF ARISTOLOCHIC ACID; RISK FOR ARISTOLOCHIC ACID NEPHROPATHY AND BALKAN ENDEMIC NEPHROPATHY

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Ingestion of aristolochic acid (AA) is associated with the development of aristolochic acid nephropathy (AAN), which is characterized by chronic renal failure, tubulointerstitial fibrosis and urothelial cancer. AA may also cause a similar type of kidney fibrosis with malignant transformation of the urothelium, the Balkan endemic nephropathy (BEN). AA is among the most potent 2% of known carcinogens. The predominant AA-DNA adduct, 7-(deoxyadenosin-N^6-yl)aristolactam I (dA-AAI), which is the most persistent of the adducts in the target tissue, is a mutagenic lesion leading to A→T transversions in the *p53* gene in DNA from urothelial tumors of AAN and BEN patients. Understanding which human enzymes are involved in AA activation and/or detoxication is important in the assessment of an individual's susceptibility to this carcinogen. Despite extensive research,

contribution of most of the enzymes found to metabolize AA *in vitro* to the development of AAN and BEN diseases is still unknown. We were able to identify the major human hepatic and renal enzymes responsible for DNA adduct formation *in vitro*. Phase I biotransformation enzymes play a crucial role in the metabolic activation of AA, while a role of phase II enzymes in this process is questionable. Most of the activation of AA in human hepatic microsomes is mediated by cytochrome P450 (CYP) 1A2 and, to a lower extent, by CYP1A1. In human renal microsomes NADPH:CYP reductase (POR) is more effective. Cyclooxygenase (COX) is another enzyme activating AA in human renal microsomes. Among the cytosolic reductases, hepatic and renal NAD(P)H:quinone oxidoreductase (NQO1) is the most efficient in the activation of AA *in vitro*. Studies with purified enzymes confirmed the importance of CYPs, POR, COX and NQO1 in the AA activation. The orientation of AA in the active sites of CYP1A1/2 and NQO1 explains the strong reductive potential of these enzymes for AA. CYPs seem to be the most important enzymes participating in AA detoxication. We hypothesized that inter-individual variations in expressions and activities of enzymes activating and detoxicating AA may be one of the causes responsible for the different susceptibilities to this carcinogen reflected in the development of AAN and BEN diseases.

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GLICLAZIDE HYPOGLYCAEMIC EFFECT IN RELATION TO A LONG-TERM TOXICITY STUDY IN NORMOGLYCAEMIC RATS

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Conducting of a long-term toxicity study with gliclazide, a second-generation sulfonylurea oral hypoglycaemic agent, brings a serious health problem caused by strong hypoglycaemic response in normoglycaemic rats. The aim of this study was to find adequately effective hypoglycaemic dose of gliclazide (in relation to adverse drug reaction as well) applicable during the 6-month toxicological study. The median lethal dose (LD_{50}) established by us was 2.6 g/kg and 1.75 g/kg in male and female rats, respectively, ranking the gliclazide among moderately toxic xenobiotics. With respect to the hypoglycaemic effect, the single and repeated dose 25 mg/kg was finally chosen from three different doses tested (5, 25 and 50 mg/kg). It corresponds to dose, which is 4times higher than the maximal therapeutic dose for man calculated to the body surface, and produced glycaemia decrease by 50–60% after single administration and by 50% after repeated administration with maximal effect 8 hours after the administration. Glucose levels were determined from a tail blood drop using a glucometer-strip system after p.o. gliclazide administration. According to these results, in the 6-month toxicity study, the daily doses 20 and 30 mg/kg of gliclazide were used. To ensure the safety of repeated administration of gliclazide over the 6 months, the previously established glucose dose was added to the drinking water (150 mg/ rat/day). After the 6-months administration of gliclazide any of the following parameters: behaviour, urine analysis, blood count, biochemical parameters (except hypoglycaemic effect in female) and histopathological examination were not affected. Over the period of administration, only increased food intake in female without changed weight gain and an increased intake of water in both sexes were observed.

OXIDATION OF CARCINOGENIC 2-NITROANISOLE AND ITS METABOLITE 2-NITROPHENOL BY CYTOCHROMES P450

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2-Nitroanisole (2-methoxynitrobenzene, 2-NA) is an important industrial pollutant. It is used primarily as a precursor in the synthesis of *o*-anisidine (*o*-methoxyaniline), an intermediate in the manufacture of many azo dyes. 2-NA exhibits carcinogenic activity, causing neoplastic transformation in the urinary bladder and, to a lesser extent, in spleen, liver and kidneys in rodents. 2-NA is oxidized by rat, rabbit and human hepatic microsomes to 2-nitrophenol (2-NP), 2,5-dihydroxynitrobenzene (2,5-DNB) and 2,6-dihydroxynitrobenzene (2,6-DNB), which are suggested as detoxication 2-NA metabolites. 2-NP is the major metabolite generated by rabbit and rat microsomes, but 2,5-DNB is the predominant product formed in human microsomes. Therefore, hepatic microsomal P450 enzymes participate in detoxication of this environmental carcinogen. Here, we show that one of the 2-NA metabolites, 2-NP, is metabolized by rat microsomes to 2,5-DNB. Treating rats with an inducer of CYPs of a 2B subfamily (phenobarbital) leads to an increase in 2-NP oxidation, while that with pregnenolone-16α-carbonitrile and β-naphoflavone had lower effects. Most of inhibitors of individual CYPs tested in this study, α-naphtoflavone (α-NF for CYP1A), diamantane (DIA for CYP2B), sulfaphenazole (SULPH for CYP2C), quinidine (QUI for CYP2D), diethyldithiocarbamate (DDTC for CYP2E1) and ketoconazole (KETO for CYP3A) influenced the 2-NP metabolism. DDTC, CHIN, KETO, DIA inhibit generation of 2,5-DNB, whereas α-NF and SULPH were almost without this effect. Using microsomes of Baculovirus transfected insect cells containing recombinant human or rat CYPs and NADPH:CYP reductase (Supersomes ™) we found that human CYP2E1 followed by CYP2B6, 2A6 and 3A4 were the most effective in 2-NP oxidation, while CYP2E1 followed by CYP2C11, 1A1, 2B1 were

the most efficient rat enzymes metabolizing 2-NP to 2,5-DNB. Results are interpreted from the point of view of individuals' susceptibility to carcinogenic 2-NA and its metabolites.

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ACUTE TOXICITY OF BINARY MIXTURES: ALTERNATIVE METHODS, QSAR AND MECHANISMS

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Express tests for acute toxicity determination of chemicals and their mixtures using oligochaeta *Tubifex tubifex* or using primary rat hepatocytes make it possible to obtain sufficient amount of data on toxic indices EC50ies even of binary chemical mixtures. An analysis of R-plot makes it possible to identify type of interaction among chemicals effects: aditivity, antagonism and synergism. Estimation using a function describing R-plot of both EC50 and partition coefficients resembles techniques of QSAR analysis. An example of testing a mixture of nickel chloride and diclophenac, occurring in reality in soils or waters, demonstrates a complexity of the interpretation. Their joint effect (just of these two compounds) is influenced by a mosaic of enzymes inhibition, biotransformations, binding to components of the matrix,, complex formation, formation of insoluble compounds and may be other processes. Our techniques facilitate to follow the end points. They also indicate, however, in which way a nature of the joint effect is changing in correspondence to changes of a ratio of chemicals in a mixture and with a composition of the matrix. In conclusion, it is possible easily to find an express identification of a hazard of a mixture, even to estimate a magnitude of the joint end point for each individual mixture and conditions of individual mixture, but, meantime, not to solve mechanisms of the effect.

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ANTIOXIDANTS AND Ca-ATPase ACTIVITY MODULATION AS PROTECTION AGAINST REDOX IMBALANCE IN RATS SUFFERING FROM ADJUVANT ARTHRITIS

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Adjuvant-induced arthritis (AA) is an animal model of rheumatoid arthritis (RA) in which joint inflammation is triggered by a single intradermal injection of an immunostimulatory agent (adjuvant). Redox imbalance contributes to the pathogenesis of chronic inflammatory diseases including rheumatoid arthritis (RA) not only at the site of inflammation but they provoke also systemic damage, such as muscle dystrophies around the damaged joints and myopathies.

Oxidative stress is tightly associated with imbalance of calcium homeostasis and calcium pumps play a central role in calcium signaling. The inflammatory environment of the joint in RA probably contributes to the changes in intracellular Ca^{2+} and results in the development of the chronic phase of the disease.

To induce AA, we administered intradermally *Mycobacterium butyricum* (MB) to the base of the tail of Lewis rats. Injury of sarcoplasmic reticulum (SR) from skeletal muscles of hind paws and oxidative damage of plasma were analyzed in the chronic phase of AA on day 28 after MB injection.

Increase of protein carbonyls analyzed by ELISA and lipid peroxidation was observed in plasma. Antioxidants of pyridoindole structure had a preventive effect against protein carbonyl formation in plasma, however they did not affect lipid peroxidation. Neither protein carbonyl increase nor lipid peroxidation measured by MS was observed in SR of rats suffering from AA. Elevation of Ca-ATPase activity was the only alteration found in SR vesicles of AA rats and was probably caused by conformational changes of ATPase. Modulation of ATPase activity may be a signal for adaptive mechanisms resulting in suppression of free radical formation by mitochondria. Antioxidants reduced the elevated Ca-ATPase activity and this contradictory effect on natural regulation may at least partially explain why the use of antioxidants *in vivo* is not as beneficial as expected according to *in vitro* experiments.

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OCCURRENCE OF THE RARE SYNDROMES IN SLOVAKIA

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The authors present two relatively rare syndromes in Slovak population.

A case reports of children with Goldenhar syndrome is reported. This syndrome is a congenital birth defect, which involves facial deformities. It usually affects one side of the face only. Characteristic is a particially deformed or totaly absent ear (microtia) with a chin, which may be closer to the affected ear. One corner of the mouth may be higher than the other. Various developmental lesions of the eye, which could in a rare case be completely missing are shown. The risk of having child with Goldenhar syndrome is less than 1%.

Relatively rare syndromological entity is van der Hoeve de Kleyn Syndrome characterised by autosomal

dominant inheritace. One family is described with typical signs of the syndrome, comprising of osteogenesis imperfecta, blue sclere in half of the members of the family and otosclerosis. A habitual disarticulation of the hip joint is a rare sign.

Manifestation and severity of osteogenesis imperfecta is variable. Syndrome can be rarely combined with colour blindness, progressive muscular dystrophy, epilepsy and hemophylia.

MYOCARDIAL CELL-TO-CELL SYNCHRONIZATION IS IMPAIRED DUE TO ACUTE CA^{2+}-OVERLOAD THAT PROMOTE OCCURRENCE OF LETHAL ARRHYTHMIAS, WHILE ITS ELIMINATION FACILITATES SINUS RHYTHM RESTORATION

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Abnormalities in Ca^{2+} homeostasis and acute elevation of cytosolic free Ca^{2+}, e.g. due to ischemia, reperfusion or electrolyte disturbances are implicated in occurrence of ventricular fibrillation (VF) in both experimental and clinical settings. Since gap junction connexin channels ensure cell-to-cell electrical signal propagation and myocardial synchronisation we hypothesize that Ca^{2+} overload likely impairs intercellular coupling rendering the heart prone to lethal arrhythmias. Direct measurement of cytosolic free Ca^{2+} ($[Ca^{2+}]i$) in whole heart preparation is still rare and correlation of $[Ca^{2+}]i$ changes with subcellular injury is missing. Aim of this study was:1) to monitor $[Ca^{2+}]i$ in isolated guinea pig heart subjected to hypokalemic perfusion to induce VF followed by normokalemia with stobadine to restore sinus rhythm and 2/ to examine, whether $[Ca^{2+}]i$ abnormalities are associated with subcellular and cell-to-cell junctions alterations.

The findings showed that Ca^{2+}-overload induced by hypokalemia was linked with subcellular injury and impairment of cell-to-cell coupling prior occurrence of VF. Sustaining of VF deteriorated these changes. In contrast, the restoration of basal $[Ca^{2+}]i$ levels due to normokalemic perfusion with stobadine preceded the reversion of VF to sinus rhythm. Ultrastructure examination of the myocardium at the moment of sinus rhythm restoration revealed synchronised contraction of major population of cardiomyocytes likely due to recovery of cell-to-cell coupling.

The findings indicate that abnormal $[Ca^{2+}]i$ elevation is likely implicated in impairment of intercellular communication that is critical in triggering and sustaining of VF, while elimination of Ca^{2+}-overload linked with improvement of cell-to-cell coupling facilitate VF termination. These mechanisms may be involved in occurrence and/or termination of lethal arrhythmia in clinical conditions as well.

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THE INFLUENCE OF VEGETARIAN DIET ON IMMUNE RESPONSE IN ELDERLY WOMEN

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This research was the part of the comprehensive study of genetic, immune and nutritional aspects of ageing in vegetarian population.

Our study population consists of the group of 35 non-vegetarian and 34 vegetarian elderly women. Following immune assays were determined: Proliferative activity of T- and B- lymphocytes stimulated with mitogens and antigens; phagocytic activity and respiratory burst of leukocytes; and natural killer cell activity.

Statistical analysis of parameters of specific cellular immune response displayed significant differences between elderly women eating vegetarian and non-vegetarian diet. Immune function assays showed significant suppression of T-lymphocyte proliferative capacity in peripheral blood cell cultures derived from vegetarians in comparison with omnivore elderly women. Differences were most significantly pronounced in cell cultures stimulated *in vitro* with mitogens phytohemaglutinin ($p<0.001$) and concanavalin A ($p<0.01$). Proliferative capacity of T-dependent B-lymphocytes to pokeweed mitogen and non-stimulated cells was comparable in populations eating different diets. On the other hand, T-response through the T-cell receptor in vegetarian elderly women was significantly increased ($p<0.001$).

Moreover, vegetarian population had significantly suppressed phagocytic activity of granulocytes ($p<0.01$) and respiratory burst ($p<0.01$). No marked effect of vegetarian diet was seen in phagocytosis of monocytes.

Functionality of natural killer cells was moderately affected by vegetarian diet. Meanwhile percentage of lysis of non-stimulated NK-cells did not differ, in cells stimulated with interlekin-2 was decreased NK-activity found in elderly vegetarian women ($p=0.061$).

In conclusion, our data indicate that vegetarian diet might have possible impact on human immune response.

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EXPERIMENTAL APPROACHES TO STUDY EFFECTS OF HYPOXIA/ISCHEMIA ON EMBRYOFETAL AND POSTNATAL DEVELOPMENT

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Oxygen deficiency accompanied by oxidative stress is a well-known teratogenic factor. When hypoxia/ischemia

acts at the early stages of organogenesis, it represents a serious risk for the development of chronic diseases, such as bronchopulmonary dysplasia, intraventricular hemorrhage, and retrolental retinopathy. The severity of malformations and organ injuries depends on the intensity of the insult, on its duration, and on the developmental stage at the time of its occurrence. The period at the end of pregnancy and around delivery represents a sensitive developmental time window, especially related to the brain, the most vulnerable organ to oxygen supply impairment and oxidative stress. Functional maldevelopment of the brain can result in neurological, behavioral and mental disorders, such as cerebral palsy, mental retardation, attention-deficit hyperactivity disorder and/or affective disorders. The presentation will review the most frequently used experimental approaches in studying adverse effects of developmental hypoxia/ischemia. Moreover, selected results on pharmacological intervention of hypoxia/ischemia consequences will be highlighted.

Shortly, rodent models have long been established in the study of chronic intrauterine hypoxia and in peri- and neonatal hypoxia/ischemia. They have been found suitable for acute (ligation of carotid arteries in neonatal rats, exposure of neonatal rats to 100% nitrogen atmosphere or so-called "non-sophisticated" model of perinatal asphyxia based on exposure of the uterus to water bath on day 20 of gestation) as well as chronic studies (ligation of uterine arteries, phenytoin (PHT) induced chronic intrauterine hypoxia or systemic hypoxia of dams in hypoxic chamber). Our experimental studies showed a protective effect of synthetic pyridoindoles against embryo-fetal toxicity induced by prenatal PHT administration. In conclusion, using these models enables to elucidate the underlying mechanisms of hypoxia/ischemia concerning structural and functional injuries. Moreover, the experimental results obtained contribute to a more effective prediction, prevention and/or pharmacological intervention in undesirable consequences of pre- and perinatal hypoxia/ischemia events in neonates.

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EFFECT OF POPPY SEED CONSUMMATION ON THE POSITIVE RESULTS OF OPIATES SCREENING IN BIOLOGICAL SAMPLES

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Poppy seed is an popular substace of many traditional Slovak cakes for example poppy seed strudel, poppy seed bun, poppy seed noodle etc. We can eat quite great amount of it, sometimes more than 100 g. Existing problem in interpreting the results of opiate urinalysis in case of drug abuse arises from the natural occurrence of opiate alkaloids in poppy seed.

Is the positive result caused by drug abuse or by overeating delicious poppy seed buns?

REGULATION OF CYTOCHROME P450 1B1 UNDER PROINFLAMMATORY CONDITIONS

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The cytochrome P450 1B1 (CYP1B1) belongs among a heme-thiolate family of monooxygenases, which is involved in the metabolism of a variety of both exogenous and endogenous compounds. The expression and regulation of CYP1B1 is tissue-specific. The *CYP1B1* gene can be transcriptionally activated by polycyclic aromatic hydrocarbons, which act via the Ah receptor complex. In steroidogenic tissues like the adrenal, ovary and testes where AhR levels are low, CYP1B1 is expressed constitutively. High expression of CYP1B1 has been detected in wide range of human tumors [1]. Wide distribution of CYP1B1 and its involvement in biotransformation of xenobiotics, as well as endogenous substrates indicate its significant role in tumorigenesis and in hormone metabolism. The levels of various CYPs can be significantly modulated by proinflammatory cytokines such as TNF-α; the chronic inflammation is known to contribute to cancer development. We have recently found that TNF-α can differentially modulate expression of CYP1B1/1A1 in rat liver epithelial cells [2], and it can potentiate genotoxic effects of environmental pollutant benzo[*a*]pyrene through upregulation of CYP1B1 expression [3]. Although aryl hydrocarbon receptor AhR plays a major role in regulation of CYP1B1 expression [4], the upregulation of CYP1B1 by proinflammatory cytokine seems to suggest that other signaling pathways may synergize with AhR to induce CYP1B1 expression. In the present study, we explored several other regulatory pathways being involved either in the inflammatory response or in tissue-specific CYP1B1 regulation, including NF-κB, cyclic AMP-response element binding protein (CREB) or mitogen-activated protein kinases, in order to analyze their contribution to effects of TNF-α on CYP1B1 expression in rat liver epithelial cells.

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REFERENCES

[1] Murray G.I., *et al.* (1997) *Cancer Res* **57**(14):3026–31.
[2] Umannova L., *et al.* (2007) *Toxicol Sci* **99**(1):79–89.
[3] Umannova, L., *et al.* (2007) *Mutat Res* **640**(1–2):162–9.
[4] Nebert D.W., *et al.* (2006) *Nat Rev Cancer* **6**(12): 947–60.

CHROMIUM AND LEAD VALUES IN BIOLOGICAL MATERIAL OF EMPLOYEES OF A MILITARY REPAIR FACILITY

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The goal of this work was to examine the concentrations of chromium (Cr) and lead (Pb) of the company VOP

Novaky employees. The employees have been removing the consequences of the explosion from 2. Marc 2007. It is very likely that these metals were released into company area, as well.

The authors examined content of Cr and Pb in blood and urine of 25 men. The average age of the exposed workers was 35.52 ± 7.86 years. The used analytical method was the atomic absorption spectroscopy in graphite furnace (GFAAS). This method is able to specify the total value of Cr without separation of the benefit chromium $Cr^{III.}$ and the carcinogenic hexavalent form. The collection of biological material was made after 4 month of exposure. These values were compared to the concentrations of Cr and Pb in the blood and urine of the control group (CG): 25 persons from different areas of Slovakia. The average age of the control group was 33.64 ± 11.26 years.

The acquired concentrations of blood chromium were gently increased: 0.086 exposed vs. 0.064 μmol.l^{-1} of CG ($p<0.100$). The average concentration of Cr in urine was significantly increased: 6.098 exposed vs. 3.410 μmol. mol creatinine (kr.)$^{-1}$ of CG ($p<0.010$). The values of Pb in blood of analysed cohort were 0.166 vs. 0.135 μmol.l^{-1} of CG and Pb in urine 0.951 vs. 1.473 μmol.mol kr.$^{-1}$ of CG.

The professional lead exposure was not substantial. The moderate increased values of Cr in blood ($p<0.100$) and in urine ($p<0.010$) to control group indicated increased professional exposure of this metal.

As $Cr^{VI.}$ is carcinogen of 1. category, we find important to continue monitor this company and we recommend to repeat the analyzes after 1 year.

PRO- AND ANTI-OXIDATIVE PROPERTIES OF D-PENICILLAMINE

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The study presents results of pro- and anti-oxidative effects of D-penicillamine on hyaluronan degradation by ascorbate *plus* cupric ions.

The well established degradative system comprising high-molar-mass hyaluronan and ascorbate *plus* Cu(II) ions was used [1,2]. Primarily, the effects of replacement of ascorbic acid in this system by D-penicillamine were investigated. Then, D-penicillamine was added into the above degradative system before reaction onset or 1h after the reaction had started. To monitor hyaluronan degradation kinetics, rotational viscometry was applied.

No hyaluronan degradation occurred when ascorbate was replaced by D-penicillamine. The drug addition into the complete degradative system at the reaction onset caused a marked inhibition of hyaluronan degradation. However, the inhibitory effect turned to a pro-oxidative one within appr. 1 h.

The dual behavior of D-penicillamine on hyaluronan degradation can relate to: (i) the drug completely traps •OH radicals generated from ascorbate *plus* Cu(II) ions under aerobic conditions; (ii) thiyl radicals generated from D-penicillamine react with D-penicillamine anions resulting in novel radical-reactive species, which e.g. by reducing dioxygen molecules can generate further •OH radicals.

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REFERENCES

[1] Šoltés L., *et al.* (2007) *Carb. Res.* **342:**1071–1077.
[2] Valachová K., *et al.* (2007). *Chem. Listy* **101:**287–288.

CYTOTOXICITY OF WATER EXTRACTS FROM *PHILADELPHUS CORONARIUS* L. ON HUMAN BREAST ADENOCARCINOMA CELL LINE (MCF-7)

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Philadelphus coronarius L., family Hydrangeaceae, is popular ornamental shrub occuring in East Asia, North America, South-East Europe and Caucasus. An aqueous extract from the flowers is used in traditional medicine for the treatment of some gynaecological diseases and in homeopathy (*Philadelphi flos*).

We have tested the cytotoxic effects of water extracts from leaves and branches of *Philadelphus coronarius* L. (Hydrangeaceae). The human breast adenocarcinoma cell line (MCF-7) were treated with various doses of individual extracts (0.5–500 μg dry matter/ml) for short time (24 h) and long time (72 h). Cytotoxic activity was measured by the amount of cytosolic enzyme lactate dehydrogenase (LDH) released through impaired cell membrane into the incubation media.

Both extracts displayed dose dependent cytotoxic effect, while time dependent intensified effect was observed for leaves extract only. Branches extract was more effective then leaves one for short time treatment, whereas the highest responsiveness for leaves extract was detected after 72 h. The sensitivity of cells expressed by ED50 value was similar for both tested extracts and respective time, a slight increase in sensitivity was observed after long time treatment.

The results obtained so far demonstrate cytotoxicity of both, branches and leaves water extracts and increased effect after chronic exposure. These data will provide the basis for the future studies with isolated active substances from these extracts.

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CYTOTOXIC AND CYTOSTATIC EFFECTS OF NOVEL DNA-INTERCALATORS – 2´,2´´-[(ACRIDINE-3,6-DIYL) DIIMINO]-1,3-DIIMIDAZOLIDINE-4-ONE DERIVATIVES

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One interesting group of chemotherapeutic agents used in cancer therapy comprises molecules that interact with DNA [1,2]. Acridines are known to interact with DNA, mainly by intercalation [3]. Several derivatives of 2´,2´´-[(acridine-3,6-diyl) diimino]-1,3-diimidazolidine-4-one derivatives (DIM) with different alkyl chains (propyl-,butyl-, pentyl-, hexyl-) were synthesized and DNA binding properties of these new drugs with purified thymus DNA has been studied by UV-Vis and fluorescent spectroscopy and circular dichroism. From the titration data it was possible to assume that the intercalation is the dominant binding mode. The highest value of binding constant 1.2×10^5 M^{-1} was estimated for propyl-DIM. Cytostatic effects of all derivatives have been tested against human leukaemia HL-60 cells (MTT-assay and direct cell counting, 72-hrs treatment), and strong cytotoxicity of all derivatives has been confirmed. The highest cytostatic effect has been observed when cells were incubated with hexyl-DIM (IC_{50}=6.02±1.05 μM, 72 h). Uptake of DIM derivatives has been investigated and the fluorescence of accumulated substances has been estimated. Hexyl-DIM entered into HL-60 cells very fast and already after 30-min incubation the fluorescent complex of DIM with chromosome was noticeable. Changes of cellular morphology (dyeing of cells with propidium iodide and Hoechst 33343) indicated that hexyl-DIM was able to induce apoptosis.

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REFERENCES

[1] Martínez R., *et al.* (2005) *Curr. Med. Chem.* **12:**127–51.
[2] Janovec L., et al. (2006) *Chem.* **18:**93
[3] Aslanoglu, M. (2006) *Anal. Sci.* **22:**439–43

CHANGES IN METALLOTHIONEIN LEVEL AT RATS EXPOSED TO CISPLATIN

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Although cisplatin has been successfully used in the chemotherapy of cancer for more than 25 years, its biochemical mechanism of action is still unclear. The current accepted opinion about cisplatin mechanism of action is that the drug induces its cytotoxic properties through binding to the nuclear DNA. However, before cisplatin enters the cell, it may bind to phospholipids and phosphatidylserine in the cell membrane. In the cytoplasm many potential platinum-binding sites are also available, including sulphur-containing biomolecules, such as glutathione and metallothioneins (MT). They are a group of low molecular weight single-chain proteins rich in cysteine. The aim of this work was to monitor changes in MT level in blood serum samples from rats exposed to cisplatin.

Male Wistar rats, 8 weeks old (270–280 g), were divided into two experimental groups per six specimens. The first experimental group was exposed to one dose of 1.05 mg of cisplatin per kg, the second group to one dose of 2.1 mg of cisplatin per kg. Cisplatin was administered intraperitoneally. Metallothionein was determined by differential pulse voltammetry Brdicka reaction. Electrochemical measurements were performed with 747 VA Stand instrument connected to 746 VA Trace Analyzer and 695 Autosampler.

We collected the samples from both experimental groups per hour and determined there level of MT. We found out that synthesis of MT enhanced quickly. Even one hour after administration of cisplatin the level of MT increased at both experimental groups. Particularly, the level of MT in blood serum of untreated rats was app. 2.9 $\mu mol.l^{-1}$. At rats treated with 1.05 mg and/or 2.1 mg of cisplatin for one hour the MT level was 4.2 and/or 4.3 $\mu mol.l^{-1}$, respectively. The highest level of MT at rats treated with 1.05 mg cisplatin was determined after four hours as 4.9 $\mu mol.l^{-1}$. In the case of the second experimental group the maximum was reached even after two hours of the treatment as 4.8 $\mu mol.l^{-1}$. This phenomenon can be related with immediate triggering of MT synthesis after administration of cisplatin.

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ISOLATION AND CHARACTERIZATION OF CYTOPLASMIC NADPH-DEPENDENT PHENOL HYDROXYLASE AND CATECHOL-1,2-DIOXYGENASE FROM *CANDIDA TROPICALIS* YEAST

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Biodegradation process is based on efficiencies of various organisms (both prokaryotic and eukaryotic) to utilize xenobiotics as a source of carbon and energy for their intermediary metabolism. Such organisms are able to degrade xenobiotic compounds without formation of any secondary waste products. Therefore, biodegradations are economic as well as ecological methods. *Candida tropicalis* yeast is one of organisms capable of metabolizing phenol and utilizing it as the

only source of carbon and energy. The *C. tropicalis* yeast Ct2 was isolated from soil contaminated with aromatic hydrocarbons. Degradation of phenol in this yeast comprises of several steps, the final products being then implemented into intermediate metabolism of this organism. The enzymes responsible for the first step of degradation (the formation of catechol) are: (i) cytochrome P450 (EC 1.14.15.1), the enzyme of the mixed function monooxygenase system localized in the membrane of endoplasmic reticulum [1] and cytoplasmic NADPH-dependent phenol hydroxylase (EC 1.14.13.7) [2]. During the second step of phenol degradation, intradiol cleavage of catechol to cis,cis- muconic acid occurs, being catalyzed by cytosolic catechol-1,2- dioxygenase (EC.1.13.11.1). In this study, the isolation procedures for purification of cytosolic hydroxylase and catechol dioxygenase were developed. The procedures consists of preparation of cytosolic fraction from *C. tropicalis* yeast by centrifugation, chromatography and rechromatography on a DEAE-Sepharose column (linear gradient of 0–0.5 M NaCl was used for separation of proteins), dialysis to remove the low-molecular particles followed by gel permeation chromatography. Four types of gels were tested for their ability to separate both enzymes using gel permeation chromatography: Sephacryl S-300, Sepharose 4B, Sephadex G-75 and G-100. The partially purified enzymes were additionally purified using affinity and/or hydrophobic chromatography on a column of Phenyl-Sepharose. The enzyme activity was followed by HPLC and the purity of enzymes was controlled with sodium dodecyl sulfate (SDS)-polyacrylamid gel electrophoresis. The purified enzymes were partially characterized.

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REFERENCES

[1] Stiborová M., *et al.* (2003) *Gen. Physiol. Biophys.* **22:**167.
[2] Páca J. Jr, *et al.* (2007) *Enzyme Microb. Tech.* **40:**919.

XENOTRANSPLANTS OF HUMAN MESENCHYMAL STEM CELLS IN DIABETIC RATS

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According to the results obtained over the last a few years, human mesenchymal stem cells (hMSCs) proved an exceptional tool in regenerative medicine. Their abilities to reconstruct damaged tissues after trauma or a degenerative process are especially valuable [1]. Their immunologic properties able to inhibit an inflammation process or the immune response of the recipient are also extraordinary. According to Le Blanc [2] allogenic implants of MSCs are non-immunogenic in humans and in experimental animals, without need of immunosuppression.

The aim of the present study was to investigate (i) survival, migration, differentiation properties of hMSCs transplanted into non-immunosuppressed rats after streptozotocin induced diabetes mellitus, and (ii) impact of hMSCs transplantation on functional recovery. Two types of heterologous hMSCs were used, i.e. bone marrow derived (BMhMSCs) and fat tissue derived (FDhMSCs), both in two applications, the second seven days after the first one. There was a difference between the two types of MSCs used. The BMhMSCs decreased the postprandial glucose level of experimental rats 48 hours after the second application, while the FDhMSCs did not.

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REFERENCES

[1] Čížková D., *et al.* (2006) *Cell Mol Neurobiol* **26**(7–8):1167–80.
[2] Le Blanc K, *et al.* (2006) *Curr Opin Immunol* **18**(5):586–91.

METALS – IMPACT AND IMPLICATIONS

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Toxicological impact of selected metals is evaluated from the experimental knowledge. Implications, concerning potential mechanisms of noxious effect within relevant mammal tissues are postulated, especially those, relevant to cellular oxidative metabolism as influenced by metal presence.

In vitro cadmium is known to increase O_2^- production in human granulocytes or alveolar macrophages [1]. In two rat tissues significant influence of cadmium on oxygen consumption of intestinal and liver tissues was demonstrated together with morphological correlation [4]. Desoize [2] is including chromium, arsenic, nickel, vanadium, iron, copper and manganese as metals with action, realised by ROS production. Updated information on iron, copper, cobalt, vanadium, cadmium, arsenic and nickel reports on their capacity of free radical formation [3].

Penetration of some of above mentioned metals to the brain (as well as other organs) may implicate similar toxicologic impact. Penetrations of manganese or cadmium or lead to the brain were evaluated [5,6, unpublished results].

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REFERENCES

[1] Amoruso MA, *et al.* (1981) *Toxicol Lett* **10**(2–3):133–138.
[2] Desoize B. (2003) *In vivo* **17**(6):529–539.
[3] Valko M, *et al.* (2006) *Chemico-biol interact* **160**(1):1–40.
[4] Vojtíšek M, *et al.* (1989) *Acta Morphol Hung* **37**(3–4):201–217.
[5] Vojtíšek M, *et al.* (2005). *Electromag Biol Med* **24**:355–358.
[6] Vojtíšek M, *et al.* (2007) *Chem Listy* ***101****:* 73, 310, 296–297

Ah RECEPTOR-DEPENDENT DEREGULATION OF CELL PROLIFERATION IN LIVER PROGENITOR CELL LINE – POSSIBLE MECHANISMS

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The aryl hydrocarbon receptor (AhR) is a transcription factor involved in toxic responses to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and related compounds. Activation of the AhR by TCDD leads to its dimerization with ARNT and transcriptional activation of a number of target genes, including xenobiotic metabolizing enzymes. However, AhR apparently fulfills a number of other functions in the organism, which may explain pleiotropic effects of a number of environmental pollutants, including e.g. developmental toxicity and tumor promotion. Using an *in vitro* model of rat liver progenitor cells, WB-F344 cell line, we study effects of AhR ligands on regulation of cell proliferation, cell death and cell-to-cell communication. Disruption of contact inhibition appears to be a major mode of action of AhR ligands in WB-F344 cells, which seems to involve induction of the transcription factor JunD and transcriptional upregulation of cyclin A. The disruption of contact inhibition depends on presence of functional AhR, but it probably does not require ARNT. Nevertheless, our recent results seem to suggest that numerous other cellular components are deregulated via AhR activation as well, including disruption of gap and adherens junctions, and a number of signaling pathways that are involved in liver cell control, as indicated by the results of microarray analysis of alterations in gene expression upon the treatment with a model AhR ligand, 3,3',4,4',5-pentachlorobiphenyl (PCB 126).

This work was supported by the Czech Science Foundation, grant No. 524/06/0517.

COMPARISON OF THE SENSITIVITY OF *DANIO RERIO* AND *POECILIA RETICULATA* OF SILVER NITRATE IN SHORT-TERM TESTS

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The aim of the study was to assess the acute toxicity of silver nitrate to adult zebrafish and adult guppies and compare the sensitivity of these species to this compound. Silver is a naturally occurring element and it is found in the environment combined with other elements such as sulfide, chloride, and nitrate. Silver presented as silver nitrate is one of the most toxic metals to freshwater fish. An industry, particularly photographical and electrotechnical, is the major source of silver that is released into the environment; it is used in electrochemical plating, in chemical industry and in health service.

The tests of acute toxicity were performed on the most frequently used species of aquarium fish *Danio rerio* and *Poecilia reticulata*. International guidelines recommend these fish as the standard organism for toxicity tests. The main advantages of zebrafish and guppies include their economical husbandry, small yet accessible size, high reproductive capacity, genetic tractability, and a large and growing biological database.

Both zebrafish and guppies were exposed in progressive concentrations series of silver nitrate; the semistatic method according to OECD 203 was used in the tests. In each test series, 5 tests of acute toxicity were made, with 10 fish used for each concentration and for the control group. The results (number of fish death in individual test concentrations) were subjected to the probit analysis (EKO-TOX 5.1 software) to determine the 96hLC50 $AgNO_3$ values. Statistical significance of the difference between LC50 values in guppies and zebrafish were evaluated by using the Unistat 5.1 programme.

Comparison of the 96hLC50 silver nitrate values in zebrafish and guppies indicates that guppies are more sensitive to silver nitrate acute exposition than zebrafish.

The work was realized with the support of MSM Project No. 6215712402 Veterinary Aspects of Foodstuff Safety and Quality.

CHELERYTHRINE AND DIHYDROCHELERYTHRINE INDUCE APOPTOSIS, NECROSIS AND CELL CYCLE ARREST IN HUMAN LEUKEMIA HL-60 CELLS

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Chelerythrine, a member of quaternary benzo[*c*] phenanthridine alkaloids, shows a variety of biological activities including cytotoxicity against normal and cancer cells. On the contrary, little is known about the biological activity of dihydrochelerythrine, a product of chelerythrine reduction. We compared cytotoxic effects of chelerythrine and dihydrochelerythrine in human promyelocytic leukemia HL-60 cells. As determined by MTT reduction assay, a 4 h treatment of HL-60 cells with chelerythrine resulted in a dose-dependent decrease in the cell viability with IC_{50} value of 2.6 μM. Dihydrochelerythrine was found to be less cytotoxic since the viability of cells exposed to 20 μM dihydrochelerythrine for 24 h diminished only to 53%. Decline in the cell viability induced by both alkaloids was accompanied by apoptotic events including loss of the inner mitochondrial membrane potential, increase in the caspase-9 and -3 activities, and appearance of cells with sub-G1 DNA. Chelerythrine, but not dihydrochelerythrine, elevated the activity of caspase-8 as well.

Using annexin V/propidium iodide dual staining flow cytometry, we observed that chelerythrine and dihydrochelerythrine induced both apoptotic and necrotic mode of cell death. Moreover, both alkaloids appeared to cause accumulation of HL-60 cells in G1 phase of the cell cycle. We conclude that both chelerythrine and dihydrochelerythrine affect cell cycle distribution, activate intrinsic apoptotic pathway, and induce bimodal cell death in HL-60 cells.

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INTERESTING TOXICOLOGICAL CASES

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Intoxications by drugs, medicaments or agrochemicals might, by insufficiently fast (late) first aid and diagnostics, endanger the health and life of the affected person.

The intoxications may be caused:

- on purpose (drug abuse, alcohol abuse or their combinations, suicidal activity),
- due to lack of knowledge (mostly at children),
- by the chemical disasters,
- unfortunate coincidence, mistake, replacement.

Intoxication diagnosis might be difficult if a clinical view is not typical one. It is primarily based on the anamnesis and the only laboratory toxicological examination is diagnosis confirmation. Numerous methods of analytical chemistry mainly immunochemical and chromatographical methods are used for the identification and quantitative analysis of toxic substances in biological materials. At our poster we depict three interesting toxicological cases, out of which two ended up in death.

DEREGULATION OF CELL-TO-CELL COMMUNICATION IN RAT HEPATOMA CELLS BY TCDD AND PCB126

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2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) and 3,3´,4,4´,5-pentachlorobiphenyl (PCB126) are considered to be environmental carcinogens, which toxic and tumor promoting effects are mediated predominantly through the aryl hydrocarbon receptor (AhR) signaling pathway. Deregulation of cell proliferation, cell cycle control and cell-to-cell communication are believed to be involved in tumor promotion; and, together with e.g. increased cell motility, they enable cells to escape control mechanisms maintaining tissue homeostasis and may contribute to tumor invasivity. 5L and BP8 cell lines, both selective variants of H4IIE cells, represent a powerfull tool for elucidating the role of AhR in the processes ocurring in rat hepatocellular carcinoma. We therefore used these two cell lines and tested the ability of model AhR ligands, PCB126 and TCDD, to induce changes in localization and/or expression patterns of proteins involved in cell-to-cell communication, especially in cadherin-, catenin- and integrin-mediated junctions. We found that TCDD- and PCB126 induced deregulation of intercellular communication, which seemed to be predominantly AhR-dependent, as we did not detect any significant changes in catenin, cadherin or integrin expression in AhR-deficient BP8 cells. These data thus reveal a significant impact of environmental pollutants (represented here by TCDD and PCB126) on deregulation of cell-to-cell communication and a possible biological importance of AhR signaling pathway in the processes of liver carcinogenesis.

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