ABSTRACTS

(in alphabetical order)

Note: The authors are solely responsible for the scientific content and linguistic presentation of the abstracts.

ELLIPTICINE AND BENZO(A)PYRENE INCREASE THEIR OWN METABOLIC ACTIVATION VIA MODULATION OF CYP1A1/2 ACTIVITY

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Two compounds known to covalently bind to DNA after their activation with cytochromes P450 (CYPs), benzo(a)pyrene (BaP) and an antineoplastic agent ellipticine, were investigated for their potential to induce CYP and NADPH:CYP reductase (POR) enzymes in rodent livers. For both compounds, hepatic POR-null (HRN™) mice and wild-type littermates were used as animal model; the potential of ellipticine to influence the CYP1A expression and DNA adducts formation *in vivo* was also evaluated in rats, the animals found to be suitable to mimic the fate of ellipticine in humans.

Both compounds induce expression of CYP1A1 and 1A2 enzymes in rodent livers, the main target organ for DNA adduct formation. Levels of ellipticine-derived DNA-adducts formed *in vivo* in the livers of HRN™ mice were reduced (by up to 65%) relative to levels in WT mice, indicating that POR-mediated CYP enzyme activity is important for the oxidative activation of ellipticine to metabolites generating these adducts. In contrast to these results, 6.4-fold higher DNA binding of BaP was observed in the livers of HRN™ mice than in WT mice.

When liver microsomal fractions were incubated with ellipticine or BaP, DNA adduct formation in calf-thymus DNA, measured by ^{32}P-postlabelling analysis, was up to 25-fold higher in incubations with microsomes from pretreated animals than with controls. The observed stimulation of DNA adduct formation was attributed to induction of CYP1A1/2 enzymes, which are responsible for oxidative activation of ellipticine to 13-hydroxy- and 12-hydroxyellipticine or that of BaP to benzo[*a*]pyrene-7,8-dihydrodiol-9,10-epoxide (BPDE), the metabolites generating major DNA adducts by both compounds. Taken together, these results demonstrate that by inducing CYP1A1/2, ellipticine and BaP increase their own enzymatic metabolism leading to an activation of these xenobiotics to reactive species forming DNA adducts, thereby modulating their either pharmacological (ellipticine) and/or genotoxic potential (both chemicals).

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FLEXIBILITY OF CYTOCHROME P450 ACTIVE SITES AS A NECESSARY CONDITION OF THEIR FUNCTION

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Cytochromes P450 are the key enzymes in the majority of reactions leading to detoxication and metabolism of xenobiotics in the man. Experimental (UV VIS absorption under high hydrostatic pressure, and resonance Raman spectroscopy) and theoretical (molecular dynamics simulations) lead to the conclusion that cytochromes P450 differ significantly in malleability of their structures, namely, in the flexibility of their active sites. Among the human liver microsomal cytochromes P450 (CYPs), the CYP3A4 is the most flexible enzyme which nicely corresponds to its ability to bind the majority of drugs with known CYP-mediated metabolism. On the contrary, the enzymes with relatively narrower substrate specificity exhibit a more rigid structure with the active site less flexible. Also, the denaturation of CYPs, i.e. the conversion of native enzyme to an inactive, cytochrome P420 form, is the most easy in the more flexible CYP enzymes. In some CYPs, the substrate binding leads to a significant increase in the stiffness of the active site.

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ALLERGENICITY TESTING USING PLASMACYTOID DENDRITIC CELLS

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An *in vitro* predictive test system for assessing the allergenicity potential of substances will have utility throughout industry to monitor products for contact allergenicity. Development of such non-animal alternative assay systems for skin sensitization hazard assessment is within the provisions of the European Union chemicals policy known as REACH (Registration, Evaluation, and Authorization of Chemicals). We investigated whether phenotypic and functional changes to subset of dendritic cells (DC), plasmacytoid DC (pDC), could be used to identify allergens. To achieve this goal, normal human DC were generated from CD34+ progenitor cells and cryopreserved. Frozen DC were thawed and the pDC fraction (CD123+/CD11c–) was harvested using FACS sorting. The pDC were cultured, expanded, and pulsed with chemical allergens (n=13) or irritants (n=7). Subtoxic concentrations of each chemical were determined using FACS analysis of propidium iodide stained cells. Results showed that exposure of pDC (n=2–5 donors) to allergens induced an increased (~ 1.5 fold) expression of CD86 for 12 of 13 allergens tested. On the other hand, 7 of 7 non-allergens did not result in increased CD86 expression. Based on these results, a preliminary prediction model was developed to identify chemical allergens (sensitivity=91–93% and specificity=93–100%). In conclusion, CD86 expression in pDC appears to be a sensitive and specific predictor of allergenicity of chemicals. When compared with existing animal models, the assay is advantageous because high throughput screening of chemicals using cells of human origin is possible at low cost.

MAGNETIC RESONANCE SPECTROSCOPY (MRS) AND IMAGING (MRI) OF EXPERIMENTAL HYPOXIA-ISCHEMIC DAMAGE TO THE BRAIN

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The aims of this study were (i) to find non-invasive parameter reflecting cerebral ischemia, and (ii) to evaluate the effect of a new drug (pyridoindole derivate) in brain ischemia by *in vivo* MRS and MRI. All experiments were performed at 4.7 T SISCO 200/330 imaging spectrometer with 100 mT/m gradient insert. Bilateral occlusion of the left and right carotid communis (12 min) with subsequent reperfusion under halotan anesthesia was performed on 7–9 weeks old male gerbils. NMR measurements were performed 30 min after the onset of reperfusion. *In vivo* ^{31}P MR spectra were collected using surface radio-frequency coil with a typical line width of 40–80 Hz in proton signal. To assess creatine kinase forward rate constant (k_{for}), a sensitive indicator of various pathological states, saturation transfer measurements were accomplished by DANTE pulse sequence [1]. Diffusion-weighted imaging with the following settings TR/TE=2500/50 ms, diffusion gradient duration (d)=8 ms, diffusion time (D)=35 ms, b=0.15, 15, 60, 133 and 370 s/mm^2, and T_2-weighted imaging TR=2500 ms, TE=30, 50, 90 and 120 ms, according to [2], were performed on the global ischemia model on gerbils.

In the applied model of ischemia, we found significant decrease of k_{for} in the ischemic group relative to the control one ($p<0.001$). This finding is in agreement with the hypothesis on the impaired energy metabolism due to hypoxic-ischemic insult. Further experiments are needed to assess effect of novel drugs. Diffusion- and T_2-weighed imaging reflected well the ischemic changes. However, the observed ischemia-induced lesions were not sufficiently reproducible. Improvement of experimental design is suggested.

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EXPERIMENTAL STUDIES, PRACTICAL IMPLICATIONS AND PERSPECTIVES IN THE PROPHYLAXIS AGAINST NERVE AGENT POISONING

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Approaches to prophylaxis against nerve agent poisoning are based on different principles: keeping acetylcholinesterase (AChE, key enzyme for toxic action of nerve agents) intact (protection of AChE) is a basic requirement for effective prophylaxis. It can be reached by using reversible inhibitors (preferably carbamates), which are able to inhibit AChE reversibly. AChE inhibited by carbamates is resistant to nerve agent inhibition. After spontaneous decarbamylation, normal AChE serves as a source of the active enzyme. Detoxification, i.e. administration of the enzymes splitting or binding (cholinesterases) the agent can be used. The agent is bound to the exogenously administered enzyme and thus, its level in the organism is decreased ("scavenger" effect). The administration of enzymes (AChE and butyrylcholinesterase, BuChE) as scavengers seems to be very promising: the enzyme is acting at the very beginning of the toxic action, without interaction with target tissues and without side effects. The antidotes currently used for the treatment of nerve agent poisoning can be tested as prophylactics. This principle can be considered as simulation of treatment or a treatment "in advance". Standard antidotes (i.e. anticholinergics, reactivators, anticonvulsants and others) were studied in this respect. The problem with their use is the timing, duration and achievement of sufficient levels of these antidotes after the administration. At present, PYRIDOSTIGMINE is common prophylactic antidote in many armies. Prophylactics PANPAL (pyridostigmine, trihexyphenidyle and benactyzine) and TRANSANT (transdermal patch containing HI-6) are means introduced into the Czech Army. Future development will be focused on scavengers, and to other drugs either reversible cholinesterase inhibitors (e.g. huperzine A, physostigmine, acridine derivatives etc.) or other compounds with the aim to improve medical protection against these agents.

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MECHANISMS OF PROTECTIVE EFFECTS OF ENRICHED ENVIRONMENT INVOLVE GENDER-DEPENDENT CHANGES IN BRAIN-DERIVED NEUROTROPHIC FACTOR (BDNF) AND HORMONE LEVELS

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Environmental factors may contribute to exaggerated release of stress hormones and consequent development of cardiovascular, metabolic, oncological and mental diseases. On the other hand, complex environmental stimuli may induce positive effects on several physiological functions and particularly on brain plasticity and neurogenesis. Mechanism of increased neural plasticity under conditions of enriched environment may include changes of hormone secretion. The aim of this study was to test the hypothesis that environmental enrichment used as a model of increased brain plasticity, induces

gender-dependent effects on neuroendocrine activity in rats. Wistar rats were housed under standard conditions or in big cages with repeated changing of environmental objects (3 times a week) for 6 weeks. Male and female rats were housed separately. Blood and tissues for hormone and BDNF analyses were sampled following decapitation. Plasma corticosterone and the weight of adrenal glands were higher in female compared to male rats. Plasma ACTH increased significantly ($p<0.05$) in male but not in female rats exposed to environmental enrichment. Testosterone levels were higher in males compared to females and increased in animals housed in enriched environment ($p<0.01$).Oxytocin concentrations in plasma were higher in female than in male rats ($p<0.001$). Oxytocin content in the posterior pituitary was not modified by any experimental condition. As expected, concentrations of BDNF in the hippocampus were significantly increased by exposure to enriched environment. Moreover, BDNF levels were higher in female than in male hippocampus and the influence of environmental enrichment was significantly more intensive in females than in males ($p<0.001$). Dimorphic changes found in ACTH and testosterone secretion may contribute to increase in neural plasticity induced by housing the rats in enriched environment.

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GENOTOXICITY OF MAN-MADE MINERAL FIBRES IN THE LUNG OF RATS

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Man made mineral fibres (MMMF) have the intrinsic potential to induce oxidative stress, inflammation, genotoxicity and carcinogenicity. Potencies for inducing these effects vary widely among the different fibres and depend on their geometry and biopersistence:

pathogenic fibres are long and thin and sufficiently biopersistent. Although this paradigm is well established and the toxicity of several fibres has been thoroughly investigated, risk assessment of fibre exposure is hampered by insufficient data required for this specific purpose.

In our study we investigated the relationship between the time dependence (48h, 1 and 3 months after fibres instillation) and genotoxicity of selected types of mineral fibres – amosite (asbestos, A), glass (GF) and refractory ceramic fibres (RCF) in alveolar macrophages (AM) of rats. We also investigated the genotoxic effect of the combined exposure (fibres+cigarette smoke – S) in these cells.

AM were isolated from the lung of exposed and control Albino Wistar rats. The AM cells were used to examine levels of DNA strand breaks (sbs) by the alkaline comet assay. Micronucleus (MN) test was used to assess clastogenic and mutagenic effects. Comets were analyzed by visual scoring of 100 randomly selected images per gel. For MN test 14,000 cells were analysed per group.

In the time dependence experiment we found a significant increase of sbs and MN frequency in animals 48 hours after the installation by A and GF ($p=0.05$); 3 months after instillation of fibres we detected significant increase of sbs in animals exposed to GF and RCF compared to control group ($p=0.05$).

In the combined exposure experiment the animals exposed to S, S+A, RCF or S+RCF showed a statistically significant increase ($p=0.05$) of frequency of MN compared to the control animals. However, significantly increased sbs were found only in animals exposed to cigarette smoke ($p=0.05$).

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ACONITINE INTOXICATION FROM THE POINT OF VIEW OF THE OCCURENCE OF HEART RHYTHM DISORDERS AND POSSIBILITY OF THERAPEUTIC INTERVENTION OF NEW SYNTHESIZED COMPOUND 44BU

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Aconitine, one of the most violent toxins inhibits inactivation of the sodium channels which causes their persistent activation resulting in heart rhythm disorders. At present, no specific antidote is known, the treatment of aconitine intoxication is just supportive.

In previous experiments we concluded that the new synthesized compound 44Bu is more efficient in suppressing aconitine-induced arrhythmias than lidocaine [1] and than propaphenone [2]. The antiarrhythmic efficiency of 44Bu compound is due to its effect on ionic currents (I_{Na}, I_{to}, I_K, I_{K1}). This effect on ionic currents is not the same in stereoisomers of 44Bu, that's why we suppose the different antiarrhythmic effect of these substances.

The aim of this work was to monitor the occurence of the given types of atrial and ventricular arrhythmias, changes of heart rhythm and changes of the SA and AV conduction in intoxicated rats in a given time interval and to evaluate of these arrhythmias disorders after administering the racemate, R-isomere a S-isomere of 44Bu compound.

According to our result it seems that S-isomere is better than R-isomere from the point of view of antiarrhythmic impact.

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EFFECT OF CHALCONES IN THE CONDITIONS OF OXIDATIVE STRESS (PILOT STUDY)

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The aim of the study was to monitor antioxidative effect of two chalcones (trihydroxychalcone and trihydroxydihydrochylcone) in the conditions of alloxan induced diabetes mellitus in experiment *in vivo*.

The animals were divided by random selection into 3 groups (n=7). The treated groups were given chalcones via intragastric sond in doses of 10 mg/kg in Avicel, the placebo diabetic group was given only the solution of Avicel. The last group was intact. Selected laboratory parameters (glucose, urea, cholesterol, antioxidative enzymes, total antioxidative capacity, malondialdehyde in serum; diuresis, total glucose and protein losses through urine) were determined in all animals. Kidney tissue and pancreas samples were taken for histopathological analysis.

We discovered a statistically significant increase of the glutathione peroxidase catalytic activity, total antioxidative capacity and a statistically significant decrease of malondialdehyde level in the treated groups compared to the placebo group. A statistically significant decrease of blood glucose level, diuresis, glucose and protein losses through urine was identified in the treated groups compared to the placebo group. The superoxiddismutase catalytic activity, urea and cholesterol levels involved non-significant changes.

Histopathological changes of kidney tissue and pancreas were evaluated as minimum and non-significant in both treated groups and in placebo group too.

The results of biochemical examination show antioxidative (and antidiabetic?) effect of both two chalcones. The results of histopathological examination correlate with them partially only. The changes in kidneys that can be evaluated histopathologically depend on the duration of diabetes and on the fact if and how diabetes was treated.

Histopathological changes in pancreas are not usually microscopically symptomatic and findings are not consistent.

GLUCOMANNAN IN PREVENTION OF OXIDATIVE STRESS AND INFLAMMATION OCCURRING IN ADJUVANT ARTHRITIS

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Oxidative stress and inflammation contribute to the pathogenesis of rheumatoid arthritis (RA) in an interactive mode. It is therefore important to study how both processes could be affected by new experimental therapies. The aim of this study was to evaluate the effects of a biologic response modifier, glucomannan (GM) isolated from *Candida utilis*, on the course of adjuvant arthritis on Lewis rats. The GM used was of molecular weight of 30 kDa.

The experiments included healthy animals as reference controls, arthritic animals without any GM administration, and arthritic animals with GM administration in doses of 5, 7.5 and 15 mg/kg b.w. The treatment involved daily oral administration of the substance from day 0, i.e. the day of immunization *(Mycobacterium butyricum* suspended in incomplete Freund´s adjuvant) to the end of the experiment – day 28.

A beneficial effect of GM was shown mainly in hind paw volume decrease. This effect was significant for all the GM doses studied. Further, decrease of the activity of the enzyme gamma glutamyltransferase (GGT) in the spleen and hind paw joint tissue homogenates, decrease of the plasmatic activity of N-acetyl-beta-D-glucosaminidase (NAGA), and finally suppression of lysozyme and peroxidase activity assessed in peritoneal fluid was observed in arthritic animals treated with GM. Moreover, the arthritis induced decreased total antioxidant status and increased plasma protein carbonyls were found to be improved. All these findings speak in favor of anti-inflammatory and antioxidant effects of glucomannan *in vivo*. In summary, the protective anti-rheumathic effect was dose-dependent, with the most effective oral dose of 7.5 mg/kg bw. The important characteristics of GM isolated from C. utilis, such as good water solubility and relatively small molecular weight, along with the observed anti-inflammatory and antioxidant effects, appear to be promising features for its prospective use as a natural agent in prevention and supplementary therapy of RA.

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QUANTUM DOT TOXICOLOGY REVIEW

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Quantum dots (QD) are nanocrystalline structures of diverse materials, most of literature reports present semi-conductor materials. After recognizing the outstanding optical properties of CdSe, CdTe QD a broad experimental research arose aimed at applying them in medical diagnostics and therapy. These applications provoked interest in their toxicology as the used metallic elements have been recognized for long as toxic substances. The majority of toxicological reports used *in vitro* methods which ascertained the expected findings of toxicity to cells caused by leakage of the toxic elements

from the otherwise chemically stable and almost water insoluble chemical compounds, if transferred into cell culture media. Actually, reports about experimental attempts to wrap the toxic nanocrystal core into an inorganic ZnS and a third organic hydrophilic non-toxic coat are frequently emerging. Most of these studies report a significantly decreased leakage of toxic material from the wrapped nanoparticles as compared with those unwrapped. At such trials another drawback emerges in that the size of the QD particles increases so that the half-times of elimination from the organism after *in vivo* parenteral application attain unacceptable values. Moreover, after parenteral application the nanoparticles accumulate in the RES, liver, spleen and kidneys where they represent a long-lived source of slowly dissolving toxic material. It has been shown that after parenteral application the key organ for the excretion of QD are the kidneys capable to excrete effectively only particles with the diameter size up to 6 nm. On the other side, nanoparticles with the diameter size smaller than 5 nm are excreted too quickly which may significantly impair the diagnostic or therapeutic aims expected at their usage.

Appreciable proportions of the published toxicological work are hardly comparable as authors used various units at describing the dose of QD used. Calls for unifying and defining internationally the dose metrics of nanoparticles, QD including, which is inevitable at the assessment of dose to effect evaluation appear.

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THE DEVELOPMENTAL ORIGINS OF CHRONIC DISEASES: TOXICOLOGICAL IMPLICATIONS

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Human epidemiological and experimental animal studies show that suboptimal environments in fetal and neonatal life exerts a profound influence on physiological function and risk of disease in adult life. The molecular, cellular, metabolic, endocrine and physiological adaptations to intrauterine nutritional conditions result in permanent alterations of cellular proliferation and differentiation of tissues and organ systems, which in turn can manifest by pathological consequences or increased vulnerability to chronic diseases in adulthood. Intrauterine growth restriction (IUGR) due to intrauterine development derangements is considered the important factor in development of such diseases as essential hypertension, diabetes mellitus, ischemic diseases of the heart, osteoporosis, respiratory, neuropsychiatric and immune system diseases.

An early life exposures to dietary and environmental exposures can have a important effect on epigenetic code, resulting in diseases developed later in life. The concept of the „developmental programming" and Developmental Origins of Adult diseases (DOHaD) has become well accepted because of the compelling animal studies that have precisely defined the outcomes of specific exposures.

The environmental pollutants and other chemical toxicants may influence crucial cellular functions during critical periods of fetal development and permanently alter the structure or function of specific organ systems. Developmental epigenetics is believed to establish "adaptive" phenotypes to meet the demands of the later-life environment. Resulting phenotypes that match predicted later-life demands will promote health, while a high degree of mismatch will impede adaptability to later-life challenges and elevate disease risk. The rapid introduction of synthetic chemicals, environmental pollutants and medical interventions, may result in conflict with the programmed adaptive changes made during early development, and explain the alarming increases in some diseases.

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USE OF BILIARY 1-HYDROXYPYRENE AS A BIOMARKER OF EXPOSURE TO POLYCYCLIC AROMATIC HYDROCARBONS IN FISH

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Fish exposed to polycyclic aromatic hydrocarbons (PAHs) accumulate only a small amount of PAHs in their tissue. Hydrophobic PAH are readily transformed into hydrophilic metabolite and excreted in bile. Bile PAH metabolite analysis provides information about the actual exposure of fish to PAH compounds and reveals the state and suitability of the aquatic ecosystems for fish.

The aim of this study was application of 1-hydroxypyrene (1-OHP) determined in the fish bile as biomarker for the assessment of aquatic ecosystems contamination by polycyclic aromatic hydrocyrbons. Other studies have identified 1-OHP as a one of the most abundant compounds present in fish bile and this metabolite is regarded as the best general indicator of PAH exposure in fish. 1-OHP is the main metabolite of pyrene, a widespread and common PAH that is generated by many pyrolitic and petrogenic industrial processes.

Fish bile samples used in this study were obtained from male chub (*Leuciscus cephalus*) caught on seven localities in the Svratka and the Svitava River (in the Brno agglomeration, Czech Republic). 1-hydroxypyrene was determined by reverse phase HPLC with fluorescence detection (λ_{ex}=364 nm, λ_{em}=384 nm) after a release of 1-OHP from conjugates by enzymatic hydrolysis (using

β-glucuronidase/arylsulphatase enzyme solution). In order to correct for differences in bile accumulation levels, normalisation of the 1-hydroxpyrene concentrations with the biliary protein concentration was elavuated. The protein concentration was determined using Bicinchoninic Acid Protein Assay Kit (Sigma – Aldrich) using bovine serum albumin.

The highest median value was found in the locality Rajhradice (136.09 ng 1-OHP/mg protein). This median values was significantly higher ($p<0.05$) than those obtained in the localities Svratka pod Brnem (119.04 ng 1-OHP/mg protein), Židlochovice (112.14 ng 1-OHP/ng protein), Bílovice nad Svitavou (97.26 ng 1-OHP/mg protein), Kníničky (94.17 ng 1-OHP/mg protein) and Svitava pod Brnem (67.70 ng 1-OHP/mg protein). There was no significant diference in 1-OHP content between the localities Rajharadice and ČOV Modřice (129.31 ng 1-OHP/mg protein). In individual locations, results of chemical monitoring (content of PAHs) and content of 1-OHP were compared.

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EXPERIMENTAL APPROACHES TO EVALUATE ACTIVITIES OF CYTOCHROMES P450 3A

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Cytochrome P450 (CYP) is a heme protein acting as the member of the mixed-function oxidase system in oxidation of various xenobiotics, (i.e. carcinogens and toxicants) as well as endogenous substrates (i.e. steroids). In eukaryotic cells, the enzyme is located in the membrane of endoplasmic reticulum. Cytochrome b_5 is another member of this microsomal system, found to influence some of the cytochrome P450 (CYP)-mediated reactions. Understanding which CYP enzymes are involved in metabolic activation and/or detoxication of xenobiotics and endogenous compounds is important in the assessment of an individual's susceptibility to the toxic action of these substances. Therefore, investigation which of several *in vitro* experimental models are appropriate to mimic metabolism of xenobiotics in organisms is the major challenge for research of many laboratories. The aim of this study was to evaluate the efficiency of different *in vitro* systems containing individual enzymes of the mixed-function monooxygenase system to oxidize two model substrates of CYP enzymes of a 3A subfamily, exogenous and endogenous compounds, α-naftoflavone (α-NF) and testosterone, respectively.

Five systems containing CYP3A enzymes were utilized in the study: (i) human hepatic microsomes rich in CYP3A4, (ii) hepatic microsomes of rats treated with a CYP3A6 inducer, rifampicine, (iii) microsomes of Baculovirus transfected insect cells containing recombinant human CYP3A4 and NADPH:CYP reductase with or without cytochrome b_5 (Supersomes^{TM}), (iv) membranes isolated from of *Escherichia coli*, containing recombinant human CYP3A4 and cytochrome b_5, and (v) purified human CYP3A4 or rabbit CYP3A6 reconstituted with NADPH:CYP reductase with or without cytochrome b_5 in liposomes. The most efficient systems oxidizing testosterone to its 6β-hydroxylated metabolite were Supersomes^{TM} containing human CYP3A4 and cytochrome b_5, while low efficiency of this enzyme expressed in membranes of *E. coli* or reconstituted with NADPH:CYP reductase in liposomes were found. In the case of α-NF, the highest efficiency of Supersomes^{TM} containing human CYP3A4 and cytochrome b_5 in its oxidation to two major metabolites, *trans* 7,8-dihydrodiol- and 5,6-epoxide, was also found. The results presented in this study demonstrate the suitability of the supersomal CYP3A4 systems for studies investigating oxidation of testosterone and α-NF *in vitro*.

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EFFECTS OF VINCLOZOLIN, BISPHENOL A, AND GENISTEIN ON NUCLEAR THYROID HORMONE RECEPTORS EXPRESSION AND THEIR INTERACTION WITH HORMONE RESPONSIVE ELEMENT IN HUMAN MCF-7 CELLS

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Vinclozolin is a non-systemic fungicide of the dicarboximide group, registered for both pre- and post-harvest use on fruits, vegetables and ornamental plants to control *Botrytis spp., Sclerotinia spp., Monilia fruiticola* and *Gloeosporium spp.* Bisphenol A (BPA) is used in the production of epoxy resins and polycarbonate plastics. Genistein belongs to the isoflavone class of flavonoids, and it is also classified as a phytoestrogen.

The present study was undertaken to investigate the *in vitro* effects of vinclozolin, BPA and genistein i) on thyroid hormone binding to its cognate nuclear receptors in rat liver; ii) on expression of both nuclear thyroid hormone receptor subtypes (TRalpha1, TRbeta) in MCF-7 cells, and iii) on nuclear thyroid hormone receptors interaction with their cognate thyroid hormone responsive element (TRE) in MCF-7 cell line treated with the above compounds at concentrations ranging from 0.1 to 10 μmol/l for 24 and 72 h. The binding data were evaluated from the competition curves and the maximal binding capacity as well as the equilibrium association constant have been calculated from Scatchard´s plots. The expression of thyroid hormone receptors has been analyzed by the RT-PCR technique, and the thyroid hormone receptors interaction with their cognate TRE by electrophoretic mobility shift assay (EMSA).

The results from *in vitro* experiments suggest that these compounds may play a marked role predominantly

in modulation of both TRalpha and TRbeta expression in MCF-7 cells.

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ASPHYXIAL STATUS IN A NEWBORN DUE TO NEONATAL FORM OF CARNITINE PALMITOYLTRANSFERASE II DEFICIENCY

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Deficiency of carnitine palmitoyltransferase II (CPT II) is the most common autosomal recessive disorder of mitochondrial beta-oxidation of long chain fatty acids (LCFA). Deficiency of CPT II has a fascinating phenotypic variability. The clinical manifestation has three forms: neonatal, early onset infantile and late onset adult muscular form.

Aim: To present a patient with severe reversible asphyxial status due dysrrhythmia due to neonatal form of CPT II.

Term newborn delivered spontaneously (birth weight 3450 grams, birth length 52 cm, values of Apgar score 10/10) with good direct postnatal adaptation was presented on 2nd day of life with severe asphyxial status followed by cardiorespiratory insufficiency with circulatory failure. After prolonged resuscitation 3 hours the child was admitted to our department. Diagnosis of CPT II was confirmed (free carnitine level in blood 12.2 μmol/l; ratio (C16+C18):1/C2 was 0.760 by tandem mass spectrometry; activity of CPT II in leucocytes was 0.082 μmol/min x gram protein).

Neonatal form of CPT II deficiency is the most severe form and invariably fatal. This kind of metabolic disease is congenital, but cardiac problems are not detectable during prenatal period. Fasting in early newborn period is a main trigger of CPT II deficiency symptoms. The authors emphasize the investigation of acylcarnitine profiles and carnitine in serum in all cases of severe postnatal asphyxia and in a case of unusual newborn´s arrhythmias.

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SKELETAL FLUOROSIS FROM A POINT OF VIEW OF AN OCCUPATIONAL EXPOSURE TO FLUORIDES IN CZECHOSLOVAKIA (50-YEAR FOLLOW-UP)

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Electrolytic production of aluminium in Czechoslovakia started in the year 1953 in Žiar valley in the central Slovakia. However, till 1995 the hygienic conditions for health protection were not met in the factory. It underwent a reconstruction afterwards.

The authors demonstrate cases of occupational skeletal fluorosis (currently rare in Europe) in 14 metallurgists, which were all disclosed in foundry workers in Žiar nad Hronom to the year 2007. The occupational disease was diagnosed after 17.7 ± 7.67 years (x±SD) of exposure in the foundry.

The authors describe the clinical condition, haematological and biochemical tests (decreased level of ionising calcium was found in serum). The content of fluorides in urine was increased (254.4 ± 130.95 umol.l^{-1}). The average age of patients at the time of recognition of the professional etiology of the disease was 57.93 ± 7.95 years. Eight patients were older than 60 years. Skeletal abnormalities were evaluated by using X-ray skiagraphy, estimating the I.–III. stage of the skeletal fluorosis. Typically an increase of bone density was found, the compact part of long bones was coarsed, there were calcifications of the interosseous membrane between radius and ulna and some ossifications of the sacrospinal and sacrotuberous ligaments. Twelve patients suffered sensorimotor polyneuropathy of extremities, chronic bronchitis was found in 6 patients (two of them were smokers) and dyspeptic syndrome was found in 3.

The last occupational case was registered in the year 2001. The authors suppose that the production of aluminium with a modern technology of a better safety and protection of health in workers is the key which closed the skeletal fluorosis into history in the Czech and Slovak Republic.

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HVĚZDA – AN AGENT WITH THE COMBINED DECONTAMINATION EFFECT

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Recently, an intensive development of new decontamination agents was found due to the increasing importance of decontamination. Many decontamination agents are devoted to the decontamination of one type of weapons of mass destruction and they were developed for one type of decontamination device. However, the main effort is focused on the development of polyvalent decontamination agents which are able to remove contamination caused by all types of weapons of mass destruction. One type of polyvalent decontamination agent, called HVĚZDA, was developed in the Czech Republic. It was originally developed for primary decontamination of chemical warfare agents. It was found that HVĚZDA is able to decontaminate not only skin but also hard and absorptive surfaces contaminated by permanent chemical warfare agents. Other tests confirmed that HVĚZDA is also able to remove contamination caused by radionuclides which come from nuclear explosion or from nuclear power plant technology. The potency of HVĚZDA to

carry out disinfection was also evaluated. It was able to deactivate many kinds of microorganisms including spores. HVĚZDA was tested against microorganisms which are specific for contamination of hospitals, livestock production and food industry. The results obtained from laboratory and field tests favour this decontamination agent for the decontamination activities in the Army and Intergrated rescue system in the Czech Republic.

COMPARATIVE STUDY OF NATURAL ANTIOXIDANTS – RESVERATROL, NARINGIN AND QUERCETIN – IN CCL4-INDUCED LIVER INJURY IN RATS

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The influence of natural antioxidants resveratrol, naringin and quercetin on carbon tetrachloride-induced liver injury was studied in experiment in rats. Male Wistar rats (160–170 g b.w.) were treated orally once daily for 3 days with resveratrol (7.5 mg/kg b.w.), naringin (22.5 mg/kg b.w.) and quercetin (11.25 mg/kg b.w.) dissolved in 0.5% methylcellulose. One hour after the last dose of antioxidants, carbon tetrachloride (CCl_4, 0.5 ml/kg b.w., in sunflower oil) was administered by gastric gavage. Twenty-four hours after the CCl_4 administration the alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were measured in serum. The lipid peroxidation (LP), reduced glutathione level (GSH), activities of catalase (CAT) and glutathione peroxidase (GSH-Px) were estimated in liver homogenates.

The administration of CCl_4 induced an increase in serum activity of ALT (to 262%, $p<0.001$), AST (to 180%, $p<0.001$), GSH level (to 125%, $p<0.001$) and LP (to 175%, $p<0.01$) compared to control group. The CCl_4 administration inhibited the GSH-Px activity (to 83%), the CAT activity was unaffected.

In comparison to CCl_4-only treated group the pretreatment with resveratrol, naringin and quercetin ameliorated the increase of ALT activity ($p<0.005$). Resveratrol and naringin pretreatment decreased the AST activity ($p<0.005$). CCl_4-induced elevation in LP was improved only by resveratrol pretreatment ($p<0.05$). The GSH level and the GSH-Px activity remained unchanged by antioxidants pretreatment.

After antioxidants-only administration, the levels of parameters of hepatotoxicity (ALT, AST) and of oxidative stress (LP, GSH, CAT) were equal to levels in control group. The GSH-Px activity was decreased ($p<0.005$).

The data suggest resveratrol, naringin and quercetin might be used as efficient protective agents for CCl_4-induced acute hepatotoxicity. With regards to the influence on lipid peroxidation, resveratrol appears to be the most effective of used compounds.

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COMPARISON OF THE EFFECT OF EXPOSURE TO AMOSITE ASBESTOS AND THEIR SUBSTITUTES ON THE ACTIVITY OF CHOSEN ENZYMES OF RAT BRONCHOALVEOLAR LAVAGE FLUID – DOSE AND TIME DEPENDENCE STUDY

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Aim of this work was to compare the impact of exposure to glass fibres (GF) and refractory ceramic fibres (RCF) used as substitutes of asbestos on lactate dehydrogenase (LDH), acid phosphatase (ACP) and cathepsin D (CATD) activity in cell-free bronchoalveolar lavage fluid (cfBALF) and activity of LDH in blood plasma of male Wistar rats with that of amosite asbestos (A). The fibrous dusts were applicated by intratracheal instillation in the form of suspension in saline solution. The control groups were instilled by saline solution. Four doses of fibrous dusts – 0.5; 1; 2; and 4 mg were used in a two week dose dependence study (n=12 for control group, n=6 for exposed groups). The four mg dose was chosen for the 2; 30 and 90 day time dependence study (n=6 for all groups.). After exposure the animals were exsanguinated in anaesthesia and the bronchoalveolar lavage was carried out.

Activity of LDH in cfBALF did not shown dose dependence. Activity of ACP positively correlated with the dose of A and GF while the activity of CATD positively correlated with the dose of all of the examined fibrous dusts. The most expressive correlation was found after exposure to A.

Negative correlation between the activity of LDH in cfBAL and the time of exposure was found after exposure to all of tested fibrous dusts. Activity of CATD positively correlated with the time after exposure to A and RCF. No time dependence was found for the ACP activity.

The similarity of dose and time dependence curves and correlations and only a few differences found between the values of mentioned parameters after exposure to A and after exposure to GF and RCF indicated that the impact of exposure to GF and RCF in these experiments was comparable with that of amosite.

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GASTROTOXICITY OF NON-STEREOIDAL ANTI-INFLAMMATORY DRUGS IN RELATIONSHIP TO THEIR CONSUMPTION

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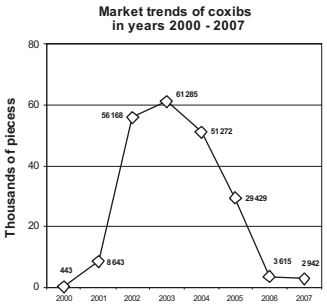
NSAIDs are ranked among most often prescribed drugs and their gastrointestinal (GI) toxicity markedly dominate among their adverse effects. Adverse GI effects

NSAIDs can hit either part of GI tract, most often gastric and duodenal mucosa, which are in per oral application of these drugs most exposed regions. Gastroduodenal (GD) mucosal lesions in patients using NSAIDs can be complicated by GI bleeding or perforation. Generality of these incidents originate without warning symptoms. Mucosal abnormalities in GI tract associated with using NSAIDs are characterized insufficient correlation within GI clinical symptoms and endoscopic finding. It was found out, that different kinds of NSAIDs to present different degree of gastrotoxicity [2]. Prescription usages concerned NSAIDs can be in individual countries effected by several factors, among them can be included also count of registered effective substances from this group, their availability, and also stage of payment of health insurance companies.

Data analysis from IMS (market trends in years 2000–2007) – ATC groups N02 and M01.

We would like to show, that with coming of the GI tract saving NSAIDs – COX 2 inhibitors decreases consumption of the drugs from the other two groups – salicylic and non-salicylic NSAIDs. This can lead to decreasing of the incidence hereinbefore mentioned complications of the treatment by NSAIDs.



The results of the analysed datas of market trends show that in years 2002 – 2004 increased consumption of selective COX-2 inhibitors, mainly in 2003 and in this year continued decreasing consumption of others NSAIDs. This process had the peak in 2004. When the COX-2 inhibitors were practically removed from market (in 2005), there is measurable growth consumption of the non-selective NSAIDs(growing more then 1 million packs in years 2005 to 2007), and as show Rybár in his two trials we can also observe increasing incidence of the adverse effects related with using of NSAIDs.

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BIOSENSORS FOR AFLATOXIN ASSAY

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The group of aflatoxins includes several difuranocoumarin derivates metabolically produced by several *Aspergillus* species (such as *A. flavus, A. parasiticus, A. niger*) in a polyketide pathway. The term aflatoxin is an acronym of the following words: ***Aspergillus fla*vus toxin**. The most widely aflatoxins (AF) are: B1, B2, G1, and G2 appointing at fluorescence under UV (B~blue; G~green). Rare derivates are e.g. M1, M2 (~milk), P1, Q1, B2A.

The most common toxicology effect is towards hepatocytes, when partial oxidation by cytochrome P450 leads to more toxic derivate obtaining 8, 9 – epoxide. Partial suppression of aflatoxin toxic effect in liver is due to glutathion S-transferase. Further effects towards biomolecules including DNA were described for non-modified aflatoxins.

The presented work is aimed at preparation and application of biosensors based on recognition capability of some biological macromolecules such as enzyme acetylcholinesterase and antibody. Electrochemical biosensors containing just mentioned macromolecules were used for assay of standards as well as real samples containing aflatoxin B1 as representative of the most frequently occurring aflatoxin.

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THE STUDY OF ANTIOXIDANT ACTIVITY OF GLUCOMANNAN ISOLATED FROM *CANDIDA UTILIS* BY CHEMILUMINESCENCE *IN VITRO* AND IN RATS WITH ADJUVANT ARTHRITIS

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Glucomannan (GM), a natural polysacharide isolated from the yeast *Candida utilis,* has been reported to possess a broad range of protective activities, including antioxidative effect.

Reactive oxygen metabolites (ROM) generation by neutrophils play a crucial role in the initiation and propagation of inflammatory responses, while various forms of antioxidative therapy may reduce pathologic inflammatory conditions. We tested the effect of GM on ROM generation in human neutrophils *in vitro* and in rats with *Mycobacterium butyricum* induced adjuvant arthritis (AA), using the luminol/isoluminol-enhanced

chemiluminescence (CL) method. *In vitro,* GM (100 and 500 μg/ml) significantly decreased PMA (4β-phorbol-12 β-myristate-α13acetate; 0.05 μmol/l) stimulated CL in human whole blood, while spontaneous CL was not affected. To specify the site of action of GM, we evaluated its effect on extra-and intracellular ROM generation in isolated neutrophils. GM signifigantly decreased spontaneous and PMA stimulated CL in neutrophils and it was more effective extracellularly than intracellularly. *In vivo* experiments included healthy animals as controls, arthritic animals without any drug administration, and arthritic animals with GM administration (once daily in the oral dose of 7.5 or 15 mg/kg, over a period of 28 days). On day 28, CL in whole blood, spleen and joint was monitored. Arthritic animals treated with GM (7.5 or 15 mg/kg) showed decrease in spontaneous and PMA-stimulated CL of whole blood as well as CL of the joint, in comparison with untreated animals. Our findings demonstrate the reduction of ROM generation by GM *in vitro* and in rats with AA, and might contribute to the elucidation of the protective effect of GM observed in diseases with an inlammatory component.

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USE OF RODENTS IN DEVELOPMENTAL NEUROTOXICOLOGY: ADVANTAGES, DISADVANTAGES AND PITFALLS IN DETECTING NEUROBEHAVIORAL DYSFUNCTIONS

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Exposure of the developing organism to industrial chemicals, drugs, hypoxia and/or stressful stimuli represents a serious risk factor for the development of neurobehavioral disorders, such as autism, attention-deficit hyperactivity disorder and mental retardation. Appropriate animal models are needed to test potential harmful effects and mechanisms of developmental neurotoxicity. In this respect, the most frequently used experimental animals are rats. Advantages of rat use in developmental neurotoxicology involve: low price of animals, low expenses for breeding, easy breeding and handling, short gestational time, numerous litters and ample scientific knowledge on physiology and behavior of rats. Disadvantages include: lack of fine neurobehavioral movements and higher cognitive function as well as differences in anatomy and time shift in brain development to postnatal period compared to humans. The developmental time shift is however advantageous for studies of the immature brain. Rat fetuses and pups can be extremely resistant to some adverse environmental factors, e.g. rat pups are highly resistant to anoxia/hypoxia due to metabolic adaptation of the brain. Subtle behavioral alterations are hard to detect and in many cases they remain masked. Hidden neurobehavioral dysfunctions can be revealed by using special behavioral, endocrine and/or pharmacological challenges, such as repeated behavioral testing, exposure to single stressful stimulus or drugs. Further, current neurobehavioral test protocols recommend testing animals up to their adulthood. However, some behavioral alterations such as anxiety-like behavior are manifested in senescence. In conclusion, our experimental and scientific experiences are highly suggestive for a complex approach in testing potential developmental neurotoxicity. Strong emphasis should be given on repeated behavioral testing of animals up to senescence and on using proper pharmacological and/or stressful challenges.

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HYALURONAN INFLUENCE ON THE ONSET OF CHONDROGENIC GENE EXPRESSION DURING DIFFERENTIATION OF MESENCHYMAL STEM CELLS

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Hyaluronan (HA) is an abundant component of chondrogenic tissue. Hence, HA is used in cartilage substitutes to replace and promote repair of damage cartilage. Inflammatory and repair processes in cartilage are associated with degradation of high molecular weight HA to low molecular fragments. These fragments differ in their activity on cartilage cells compared to high molecular HA. However, the effect of different molecular weight HA on chondrogenic differentiation of mesenchymal stem cells (MSCs), used to seed a HA composed cartilage replacements, is not clear. The aim of this study was to evaluate modulation of the MSC early chondrogenesis by HA of different molecular weight.

HA of three molecular weights were applied (100, 600, and 1500 kDa) on MSC cultured in pellet system for one, two and three weeks. Chondrogenesis was evaluated by determinations of gene expression of transcription factor Sox-9 and extracellular matrix proteins collagen type II, collagen type IX, aggrecan, and COMP by Real-Time PCR and completed with histological analysis.

All the followed genes were activated during the first week of culture, while Sox-9 was also expressed in non-chondrogenic pellets, despite of HA presence. In contrast, on the day 14 the expression of chondrogenic markers was lower in pellets incubated with HA independently on HA molecular weight. However, the final analysis after three weeks of culture revealed that the expression of all markers was uniform among all the samples without significant difference. Histological analysis of the pellet sections confirmed a proceeding accumulation of glycosaminoglycans during the differentiation without any significant effects of HA at any time point.

In conclusion, HA of any tested molecular weight did not significantly modulate chondrogenesis of MSC

in pellet system. It could be speculate that HA induced a time shift in the phase of the dominant matrix protein onset which was in full compensated in the later period.

GROWTH-RETARDED FETUSES WITH ABSENT END-DIASTOLIC VELOCITY IN UMBILICAL ARTERY AND NORMAL CARDIOTOCOGRAPHY – AN ALTERNATIVE MANAGEMENT OF THREATENING CHRONIC FETAL HYPOXEMIA

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Intrauterine growth retardation (IUGR) can be associated with many fetal adverse conditions, but the most important cause of both growth restriction and poor perinatal outcome is chronic fetal hypoxemia (CFH). This condition is the consequence of placental obliterative vasculopathy affecting first the maternal-fetal supply of nutrients and later that of oxygen. These modifications are easily observable using Doppler technology. The occurrence of CFH in IUGR is about 30%, thus thorough checking and active management after IUGR recognition is required. Fetal adaptation to CFH can be studied by Doppler velocity waveform on umbilical and fetal arteries. If CFH occurs, it is possible to monitor its evolution, yielding information of clinical practical value in order to optimize the management and timing of the delivery if necessary.

The decision for preterm delivery, which may be at present one of the therapeutic options, is very difficult as eliminationof threatened CFH must be considered with regard to the risks of prematurity. A special situation may occur when cardiotocography (CTG) seems to be normal at the absence of end diastolic velocity (AEDV) in the umbilical artery. AEDV in the umbilical artery usually precedes the onset of abnormal fetal heart rate patterns, whose duration differs considerably among the fetuses.

Case report: 33-year-old primigravida at 30 weeks of gestation was referred for detailed examination of intrauterine growth retardation (IUGR) of the fetus. Detailed ultrasonographic examination was performed – IUGR, AEDV, oligohydramnion and normal fetal anatomy were revealed. CTG was normal. Indication for cordocentesis was to obtain rapid fetal karyotype and to perform cord blood gas analysis. Cordocentesis revealed normal karyotype, values of pH – 7.39, pO_2 – 2.72, pCO_2 – 4.85 were considered satisfactory. Continuation of pregnancy was decided in spite of persistent AEDV. Glucocorticoids, magnesium and vasodilatants were administered to the patient, cardiotocography monitoring was performed twice a day. At 34 weeks of gestation the occurrence of repetitive early decelerations was considered as an indication for induction of labor. Labor, delivery, postpartal analysis of umbilical blood gases, puerperium, postpartal and neonatal parameters were normal.

Up to 8% of fetuses with IUGR and AEDV may have an abnormal karyotype. Its rapid recognition by fetal blood sampling is recommended to avoid unnecessary interventions. In the case of normal karyotype simultaneous assessment of fetal umbilical blood analysis is very useful. This kind of examination is very significant, independently of the interval between cordocentesis and the onset of cardiotocographic pathology. This interval may be utilized for intrauterine treatment, exact monitoring of the fetus and for optimizing obstetric management.

PERINATAL HYPOXIA DOES NOT CHANGE CONCENTRATIONS OF BRAIN-DERIVED NEUROTROPHIC FACTOR (BDNF) AND PLASMA OXYTOCIN IN AGING

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Adverse events during the prenatal and perinatal periods have significant negative impact on the development of several physiological functions. Moreover, fetal and early life events are associated with chronic diseases in the adulthood, such as coronary heart disease, type 2 diabetes mellitus and psychiatric disorders. The mechanisms responsible for long-term consequences may involve dysfunction of neurotrophic factors engaged in brain plasticity. Indeed, concentrations of BDNF, an important determinant of brain plasticity and cellular homeostasis, were found to be reduced in several brain regions of prenatally stressed rats during early development. A reduced expression of BDNF was observed also in the brains of adult prenatally stressed animals. Concentrations of BDNF decline during the process of aging, which may underlie the learning and memory impairments observed during senescence. It is not known, whether the consequences of perinatal stress, described in the adulthood, may persist till the old age. The aim of the present study was to evaluate possible alterations in BDNF concentrations in the hippocampus in old rats which were exposed to hypoxia during the pre- and perinatal period. The models of prenatal (exposure of uteruses on the 20th gestational day to water bath for 20 min) and perinatal (exposure of pups on the 10th postnatal day to anoxia for 10 min) hypoxia were used. At the age of 18 months, samples of trunk blood and hippocampi were collected. Perinatal hypoxia failed to influence BDNF content in the hippocampus. There were no differences in plasma oxytocin and corticosterone concentrations between rats perinatally exposed to hypoxia and control animals. At the age of 18 months, long-term consequences of perinatal hypoxia were manifested only in a decreased ratio between the weight of left heart ventricle and the whole ventricular system.

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