Title of the project: The use of experimental and clinical models of metabolic processes, nutrition and pharmacotherapy for the advancement of knowledge, clinical practice and quality of life improvement

Grant Agency: Ministry of Education ***Project Number:*** 0021620820

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Starting date: 1.1. 2005 ***Duration (years):*** 7

Funds allocated for project -Kč (thousands): 34 833

Summary of 2008 results

Title of the presentation: **Nutritional and pharmacological intervention in experimental and clinical situations.**

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The influence of valproate (VA) on leukemia HL-60 cell line, treated by ionizing radiation, has revealed a radiosensitizing effect of VA based on faster apoptosis due to induction of p21 (and thus on differentiation of the cells). A study of gene expression in the rat liver myofibroblasts cultured in fibrin and collagen gels, the components of liver extracellular matrix, has shown that collagen stimulates expression of proteases able to cleave both native and denatured collagen. Non-functioning pituitary adenomas after surgery have been characterized by the Ki-67 proliferation index, growth rate, and invasiveness (in a longitudinal, still running study).

Interactions of selected signalling pathways after administration of micronutrients (selenium, zinc and inositol hexaphosphate) were studied with help of advanced cytological techniques in colon cancer cells. In our experiments the effect of external zinc supplementation on cell proliferation, cell death, and redox system was examined during 72 h treatment in tumour cell cultivated inv itro. We can observed time-dependent activation of cell stress pathways, DNA damage pathway activation and activation od caspase-dependent pathways. We studied the effect of seleno-L-methionine and Se-methyl-L-selenocysteine on proliferation, metabolic activity and apoptosis in three colorectal cell lines with different malignant potential.

In vivo experiments were focused on introduction and characterization of a model of non-alcoholic fatty liver diseases (NAFLD) as an important prerequisite to study sensitivity of fatty liver to various hepatotoxic substances and regenerative response of the liver. Biochemical markers as well as histological evaluation documented developed NAFLD in Sprague-Dawley rats fed with high-fat for six weeks. Our pilot study documented that fluvastatin can reduce the development of cholestatic liver injury induced by the common bile duct ligation model.

In vitro experiments the non toxic effect of ursodeoxycholic acid (UDCA) in the doses prom 6.25 to 500 μmol/l was tested by WST-1 assay in primoculture of hepatocytes. The concentration of 25 μmol/l and lower were found to be optimal for further studies focused on the testing of potential hepatoprotective action of UDCA. We demonstrated that *in vitro* triiodthyronine addition to cultivation medium results in opening of mitochondrial permeability transition pore and that this effect is significantly potentiated by calcium ions.

Proteomic investigation on molecular mechanisms involved in chronic anthracycline cardiotoxicity revealed numerous significant changes (e.g., in abundance of respiratory chain complexes, Mn-SOD, mt-CK, ANT and desmin). The myocardial samples taken from animals exposed to different cumulative dose of anthracycline showed interesting changes in oxidative stress parameters and rather surprising changes MMP activities. Delayed administration of dexrazoxane was still able to afford significant cardioprotection against anthracycline cardiotoxicty. The in vivo part of the study on toxic effects of single high-dose of daunorubicin was completed and the samples are currently analyzed.

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In purpose to find new treatment possibilities of muscle wasting in proteocatabolic illness we studied the effect of administration of leucine metabolites ketoisocaproate (KIC) and hydroxy-methylbutyrate (HMB) on protein metabolism in healthy and septic rats. A decrease in leucine clearance and whole-body protein turnover (i.e., a decrease in whole-body proteolysis and protein synthesis) was observed in HMB treated animals. Significant decrease in proteolysis was found in skeletal muscle, decrease in protein synthesis was observed in the heart, colon, kidney, and spleen. The effect of KIC was insignificant.

Methotrexate hepatic elimination during co-administration with amiodarone is decreased. New insight into acute amiodarone-induced hyperbilirubinemia was described, where the increased bilirubin production and decreased renal clearance may contribute to this effect. Long term methamphetamine abuse decelerated and attenuated the brain activity following visual stimuli in group of 23 abstaining abusers. Prolongation and attenuation of visual motion and cognitive brain processing observed in 15 Alzheimer patients did not change during 6 month memantine treatment.

Regarding characteristics of stem cell markers we discovered expression of human prominin-1 (CD133) in apical and apicolateral membranes of glandular epithelia. For the first time, we identified marker of neural stem cells nestin in regenerating neuromuscular spindles. Moreover, nestin expression in the regenerating skeletal muscles correlated with formation, revascularization and reinnervation of new myofibers. We also tested the effect of ionizing radiation on human dental pulp stem cells and proved that doses of 6 Gy and higher resulted in cell cycle arrest, changes in karyotype and premature senescence. Transgenic HD rats were utilized in the study of structural changes to striatal neurotoxic lesion.

We studied the biochemical and pharmacological effects of mitoxantrone combined with acetyl-L-carnitine on an experimental solid form of Ehrlich tumor in mice. The effects were evaluated based on the mean tumor weight gain or loss, the survival time, some biochemical markers and histology of the heart and liver. Moreover, we performed comparative studies of the antitumor efficacy of selected deoxysaccharides in vitro in both human and mouse tumor cells. The HPLC method for the analysis of the sugars L-rhamnose and melibiose in biological fluids was refined.

Our group continues the study of local condition for the wound healing in acute and chronic wounds. We developed the new method of crural ulcer preparation for subsequent skin auto-transplantation using hyaluronan iodine complex. We started new study, which should assess an effect of this complex on transplant skin growth prospective randomized design. We continue our study of rheophaeresis on inflammatory activity and wound healing in ischemic skin defects. We found that the decrease in peripheral blood flow during hemofiltration is dependent on albumin level and inflammation activity. We developed the model of type II diabetic rat as the model for skin wound healing.

Three groups of patients with eating disorders, anorexia nervosa and proteino-energetic malnutrition as models of undernutrition, and metabolic syndrome as an example of overnutrition, were compared with respect to parameters of lipid metabolism. All groups revealed some common changes – raised concentrations of triglycerides, free fatty acids, apoB, palmitoleic acid in all lipid classes, and increased cholesterol synthesis expressed as lathosterol/cholesterol ratio. Decreased concentration of linoleic acid in all lipid classes as probable marker of lipoperoxidation was also quite consistent change in all the groups.

Project was supported by the Ministry of Education Research Project, No. MSM 021620820.

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