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

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Summary of 2009 results

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

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During 2009, all planned results of the current research project were achieved; in total 24 original papers were published in journals with impact factor and another 13 in peer-reviewed journals. All individual working groups within the project team produced the aforementioned papers.

Cytotoxic effect of potential cytostatic drug vanadocene dichloride was compared with that of cisplatin using two distinct human cell types - healthy nonproliferating lymphocytes and quickly proliferating leukemia cells MOLT-4. Different effects of these drugs were found in spite of their structural similarity. Expression of genes involved in the liver connective tissue metabolism was studied in cultured myofibroblasts embedded in fibrin or collagen gels. This led to a pattern that resembled more closely natural cell behaviour as compared with a standard culture medium.

We evaluated the antiproliferative, cell cycle specific and proapoptotic potential of sodium selenite in HCT-116 colorectal cells with wild type p53 and its isogenic control HCT-116-p53KO cell line. We demonstrated that sodium selenite inhibits the growth and proliferation of these cell lines in a time- and dose-dependent manner, with HCT-116 cells being mores sensitive than HCT-116-p53KO. Upon sodium selenite treatment, there was increasing expression of cyclin B1, Cdc2 p34, p21 and activation of caspase-3.

In vivo experiments were focused on comparison of toxicity of 2 model hepatotoxins – thioacetamide and D-galactosamine on intact rat liver and liver affected by non-alcoholic fatty liver disease (NAFLD). Biochemical, histological and other markers showed that steatotic liver is more susceptible to toxic injury in comparison with control rats. The other aim of in vivo study was to evaluate wheather steatosis influences course of liver regeneration after partial hepatectomy. Regeneration of the liver with simple steatosis was not significantly affected as documented above all by incorporation of bromodeoxyuridine. In vitro study dealt with the effect of Telmisartan (higly selective non-peptide angiotensin II AT_1 receptor antagonist) on rat hepatocytes in primoculture. HPLC-electrospray ionisation MS/MS method in positive ion mode with collision-induced dissociation was used for identification of parent molecule and major metabolite (1-*O*-glucuronide) in culture media and cells lyophilizates.

In purpose to understand the pathogenesis of muscle wasting and decreased levels of

branched-chain amino acids (BCAA), valine, leucine, and isoleucine, in liver cirrhosis we studied under in vitro conditions the effect of ammonia on BCAA and protein metabolism in different types of muscle. The data indicate that hyperammonemia affects directly the BCAA metabolism in skeletal muscle which results in decreased levels of BCAA in the extracellular fluid. The effect is associated with activated synthesis of glutamine, increased BCAA oxidation, and decreased release of BCAA and enhanced release of branched-chain keto acids. The effect of ammonia is more pronounced in muscles with high content of white fibers.

Mechanisms of anthracycline cardiotoxicity were further investigated. Alterations in the collagen network in relationship to the expression and activity of metalloproteinases were found. Proteomic investigation revealed remodeling of the intracellular architecture of cardiomyocytes and mitochondrial alterations. Moreover, apoptosis was showed to contribute significantly to the anthracycline cardiotoxicity and a potency of dexrazoxane (an effective cardioprotective drug) to overcome it was found. Furthermore, post-treatment follow up (i.e., long-term study of the period after the cessation of chronic daunorubicin administration) was started. During the post-treatment period, even more profound decrease in the systolic function (by 62%) was observed and biochemical changes were found (e.g., a significant increase in the left ventricular GSSG content by 82%, decline in the LV complex I activity by 28 %).

We investigated the effect of acetyl-L-carnitine alone and in combination with the antineoplastic agent mitoxantrone in an animal cancer model. Furthermore, we have shown that the intraperitoneal administration of non-toxic 6-deoxyhexoses – L-fucose and L-rhamnose – suppresses the growth rate of solid Ehrlich tumour in mice and might influence its metastatic potential. Stem cell participation in tissue repair has been examined in several models. Induction of anterior tibial muscle injury in mice by local injection of cardiotoxin combined with sublethal whole-body 9Gy irradiation and followed by transplantation of bone marrow cells gave evidence that grafted stem cells were involved not only in the first phase of the skeletal muscle regeneration (as macrophages phagocytosing the necrotic sarcoplasm of degenerated muscle fibres) but also in generation of new muscle fibres. Using the second animal model, we examined in vivo homing of transplanted bone marrow cells and lin^- $CD117^+$ cells in lethally irradiated mice. Transplanted cells from the full bone marrow showed higher reparation ability than sorted cells. Lymphatic system and its organs incl. bone marrow, thymus, spleen and lymphatic infiltration of guts were the major targets for in vivo homing.

A prospective clinical study involving 69 children and adolescents with juvenile rheumatoid arthritis examined the association between two common SNPs of the methylenetetrahydrofolate reductase gene (C677T and A1298C) and methotrexate efficacy and safety under simultaneous control for both antifolate and folate status. Carriers of the 677T allele have a 3.9- fold increased risk for MTX adverse effects. This elevation may be attributed to a 55-fold augmented risk in patients with the 677TT genotype which almost exclusively combines with the 1298AA genotype. During 6 months lasting treatment of patients with Memantine, their electroencephalographic activity did not change, indicating that the Memantine slows progress of Alzheimer´s disease seen in non-treated patients.

Evaluation of variability of bile acid and drug transport in the liver and kidney during cholestatic or drug-induced liver injury. *In vivo* experiments demonstrated that i.v. amiodarone administration can significantly decrease biliary excretion of the dye. Subsequent *in vitro* transport study using rat primary hepatocytes explained the mechanism, where the specific site of the transport inhibition was the uptake to hepatocytes through the basolateral membrane. Repeated oral administration of amiodarone to rats produced also a progressive time-dependent increase in the renal elimination of rhodamine-123 as a result of induced P-glycoprotein expression.

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