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Session 2. MOLECULAR AND CELLULAR MECHANISMS OF THE PHYSIOLOGICAL ACTIVITY OF METAL IONS

APPARENT AND TRUE INTESTINAL ABSORPTION OF MAGNESIUM ARE NOT INFLUENCED BY DIETARY MAGNESIUM LEVELS IN RATS; A TOTAL MAGNESIUM AND STABLE ISOTOPE MAGNESIUM STUDY

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The magnesium is mainly absorbed in the low parts of the intestine and its absorption is not or very little controlled. Many in vitro and ex vivo works showed that the absorption of Mg occurs primarily by passive way. The aim of this study was to determine whether the Mg intake can or not influence apparent and true intestinal absorption of Mg in rats. In that aim, thirty male Wistar rats were fed for 7 days the basal semi-purified diet, containing 600 mg Mg/kg. Groups of 10 rats were then fed for 28 days the basal semi-purified diet with different levels of Mg 150, 300 and 600 mg/kg. Mg apparent and true intestinal Mg absorption as well as Mg status indices were determined at the beginning and at the end of the experiment. Our findings show that the percentage of intestinal Mg absorption was statistically similar among the three experimental groups at both stages of the experiment. In line with this are the results obtained with the stable isotope technique which show that the percentage of intestinal ^{25}Mg absorption remained also statistically unchanged amongst the three experimental groups. Consequently, both the absorbed amount and the endogenous excretion of Mg were directly proportional to the intakes of Mg. These results indicate that passive para-cellular transport of Mg is the predominant component of intestinal Mg absorption in rats at usual dietary Mg intakes. Moreover, our data confirm also that intestinal Mg absorption is not conditioned by the body status or requirement of Mg or subject to any adaptative mechanism.

IN VITRO STUDY OF METAL TOXICITY TO RENAL EPITHELIAL CELLS

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Heavy metals are important environmental pollutants. Although some of them have proved to be essential for cell growth, cadmium (Cd) or lead (Pb) present no known biological function and are extremely toxic even at very low concentration.

Cd and Pb toxicity to renal cells is investigated on Madin-Darby canine kidney cells (MDCK cell line) as a model of the distal tubule and collecting duct. Cells are grown on two-compartment filters. They are exposed to different concentrations of metal (0.1–50μM) for various times (1–24h).

Light and electron microscopies allow the observation of morphological changes on heavy metal-treated cells. Lactate dehydrogenase release indicates membrane leakage in MDCK cells and allows the comparison of cellular proliferation with or without the toxic. Nuclear Microprobe Analyses (PIXE and RBS ion microbeam techniques) are performed to determine concentrations and distributions of biological elements and toxic metals within cells. Finally, analyses of apical versus basolateral culture media by inductively-coupled plasma-mass spectroscopy (ICP-MS) lead to a complete assessment of elemental transepithelial transfers.

Preliminary results on Cd and Pb toxicity to renal epithelial cells will be presented.

CA^{2+} IONS PLAY KEY ROLE IN GS-INDUCED ACTIVATION OF HUMAN PLATELETS *IN VITRO*

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In our recent work [1] we have shown that the gramicidin S (GS) interaction with human platelets causes (depending on the GS concentration) swelling or activation of platelets. We have made a supposition that the

mechanism of the GS-induced platelet activation involves Ca^{2+} ions. In the present work we want to demonstrate new results supporting this supposition and to propose a mechanism of the GS effect on platelets *in vitro*.

Gramicidin S is a cyclopeptide antibiotic; its molecule consists of two identical pentapeptides (Val ® L-Orn$^+$ ® Leu ® D-Phe ® Pro)$_2$ connected by the "head-tail" type and has two positively charged free amino-groups of L-ornitin residua [2, 3]. In a wide variety of environments, the GS molecule exists as a very stable amphiphilic antiparallel beta-sheet structure with a polar and a non-polar surface. This rather rigid structure is stabilised by four intramolecular hydrogen bonds, involving the four amide protons and the four carbonyl groups of the Val and Leu residues [4]. The spatial structure of the GS molecule is the important condition of the GS interaction with a membrane. The GS molecule has no specific protein receptor; it interacts directly with membrane phospholipids [2, 3, 5]. At the initial stage of interaction the GS molecule is fixed on a cell membrane interacting electrostaticly by means of NH_3^+-groups of L-ornitin with negatively charged residua of phosphate acid of lipid molecules. Then the lipid-GS complex is incorporated into a membrane changing the cyclopeptide conformation. The hydrophobic part of the GS molecule formed by Val and Leu residua is of considerable importance due to its capacity to hydrophobic interaction with a membrane. The lipid-GS complexes contact both between themselves and with membrane proteins due to hydrophobic interaction that lead to phase separation of phospholipids and to formation of a "liquid" zone of neutral lipids, which is very weak barrier for ions and molecules [2–5]. Thus GS at lower concentrations increases the permeability of model and biological membranes and at higher GS concentrations it causes membrane destabilization [2–5].

EFFECTS OF HYPOMAGNESIAEMIA ON APOPTOSIS OF SERTOLI CELLS OF THE RAT TESTIS: AN ULTRASTRUCTURAL STUDY

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Hypomagnesiaemia has been shown to increase the rate of lipid peroxidation in cells. Sertoli cell has a very important role in spermatogenesis. Apoptosis has been shown to be inhibited by calcium channel blockers verapamil and nifedipine. This serves to underscore the role of calcium as a second messenger in apoptotic signaling. On the other hand, 25-hydroxycholesterol, an oxidation product of cholesterol induces apoptosis, which is potentiated by inflammatory cytokines TNF and IFN-µ. Increased lipid peroxidation would therefore affect the outcome of spermatogenesis. This could be through programmed cell death(apoptosis) or acute cell death (necrosis).

Objective of study: To investigate the effect of induced hypomagnesiaemia on the rate of apoptosis of the sertoli cell of the rat testis, with electron microscopic ultrastructural evaluation.

Materials and Methods: Two groups of adult Sprawley-Drew rats were used (1) Diuresis induced hypomagnesiaemia and (2) Controls. After 4 weeks, the testes were harvested in both groups. End-point parameters included testicular weight, testicular tissue levels of Mg, Zn, Se and Cu, Malonylaldehyde, vitamin A,C,E and superoxide dismutase glutathione peroxidase and ceruloplasmin. Apoptosis of the Sertoli cell was evaluated with transmission electron microscopy and Tunel staining.

Results: There was a significant increase in lipid peroxidation testes with hypomagnesiaemia; higher levels of malonylaldehyde and lower levels of both enzymatic and natural antioxidants. There was also significantly higher levels of apoptosis of the sertoli in association with hypomagnesiaemia, in both the electron microscopic and Tunel staining evaluation..

Conclusion: Chronic hypomagnesiaemia may lead to impaired sertoli cell function by increased apoptosis, as a result of increased lipid peroxidation. The clinical implications in male infertility are discussed.

INTERACTION OF METAL IONS WITH CELL MEMBRANES AND MOLECULAR MODELS

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Although metals are normally used by cells, low levels of those that are nonessential, excess of the essential and, particularly, the toxic can produce serious harm. The cell membrane, as a diffusion barrier, protects the cell interior. Therefore, its structure and function are susceptible to be altered as a consequence of interactions with metal ions. In order to understand the molecular mechanisms involved metal ions such as Al(III), Cu(II) and Hg(II) were made to interact with human erythrocytes, isolated toad skin and molecular models of

cell membranes. The latter, consisting of multilayers of phospholipids commonly found in the outer and inner monolayers of cell membranes, were studied by X-ray diffraction. Examinations by optical and scanning electron microscopy allowed the determination of the type of shape change induced to erythrocytes by the metal ions. Accordingly to the bilayer couple hypothesis this information indicated in which monolayers were located the different metals. Electrophysiological measurements performed on toad skin revealed the variations in the potential difference and short-circuit current responses after application of metal ion solutions, effects that interpreted the type and extent of the perturbation induced to the active transport of ions. The observed results obtained by all these methods allowed to conclude that the three metal ions altered the molecular structure of lipid bilayers, thereby modifying biophysical properties of cell membranes. However, the extent of these alterations were a function of the nature of the metal and of their concentrations.

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BIOMARKERS OF SENSITIVITY AND EFFECT ASSOCIATED WITH CADMIUM AND MERCURY TOXICITY IN HUMAN LIVER CARCINOMA ($HEPG_2$) CELLS

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Background: In the past, demonstrating the biologic significance of chemical exposure has relied significantly on assessing and identifying the pathology induced by xenobiotic compounds. However, biomarkers of sensitivity have in recent years, increasingly being used in toxicology for detecting and predicting the harmful effects of chemical agents on the health and well being of humans and other life forms. Many measurements, including changes in enzyme activity, metabolic rates, gene expression, protein concentrations and functions, and other biochemical and physiological endpoints, have been proposed or used to characterize toxicity at the cellular and molecular levels.

Aims: This research was therefore designed to assess the cellular and molecular responses of human liver carcinoma cells following exposure to cadmium chloride, and mercury chloride; and to subsequently identify the potential biomarkers of sensitivity and effect associated with the exposure to these two metal compounds.

Methods: Cytotoxicity was evaluated using the MTT-assay for cell viability, while the gene profile (CAT-Tox) assay was performed to measure the transcriptional activation of stress genes in thirteen different recombinant cell lines generated from $HepG_2$ cells.

Results: Cyctotoxicity experiments yielded LD_{50} values of 3.5±0.6, and 6.1±0.8 ug/mL upon 48 hours of exposure to mercury chloride, and cadmium chloride, respectively; indicating that mercury was more toxic than cadmium to HepG2 cells. A dose-response relationship was recorded with respect to both cytotoxicity and gene expression. Overall, nine out of the thirteen recombinant cell lines tested showed inductions to statistically significant levels ($p<0.05$). At 2.5 ug/mL of mercury chloride, the average fold inductions were 5.2±0.9, 21.4±3.9, 7.0±6.2, 6.8±1.1, 2.7±1.0, 4.5±2.0, 7.5±6.0, 2.2±0.7, and 2.5±0.3, for GSTYa, HMTIIA, *c-fos*, HSP70, CRE, p53RE, GADD153, GADD45, and GRP78, respectively. Following exposure to cadmium chloride four of these stress genes were significantly ($p<0.05$) induced (HMTIIA, HSP70, CRE, and XRE), and seven were marginally induced (GSTYa, *c-fos*, NF-kB, p53RE, GADD153, GADD45, and GRP78). No significant inductions ($p>0.05$) were observed for CYP1A1, and RARE.

Conclusions: As expected, these results indicate that metallothioneins and heat shock proteins appear to be excellent candidates for biomarkers for detecting metal-induced proteotoxic effects at the molecular and cellular levels. This research clearly demonstrates that cadmium and mercury have the potential to cause protein damage (HTMIIA, HSP70 and GRP78), cell proliferation (*c-fos,* NF-kB), metabolic perturbation (CRE), growth arrest and DNA damage (GADD153, GADD45), and apoptosis (p53RE), and to undergo Phase II metabolism in the liver (GSTYa and XRE).

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EFFECT OF METAL IONS ON CELL CYCLE

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Metallic materials are currently used as medical devices such as bone-fixing plates and screws, artificial hip joints, heart pacemakers, cardiovascular stents, etc. When these devices are implanted into our body, they may corrode and wear, releasing metal ions and debris. Though these ions and debris can cause adverse reac-

tions of surrounding tissues and organs, the mechanism of their toxicity has not been elucidated. To understand the toxicity of metal ions, cytotoxicity of metal ions has been evaluated using mammalian or human cells. In this study, the effect of metal ions on cell cycle was examined to approach the mechanism of metal ion cytotoxicity.

Cell cycle analysis was performed using flow cytometer. First, 5.5×10^4 HeLa S3 cells were cultured in 4 mL of Eagle's MEM supplemented with 10 % (v/v) FBS for 3 d before 40 mL portion of the metal salt solution in PBS(-) was added into the medium. After another incubation of 1 d, cells were treated with 0.05 % (v/v) trypsin, 0.2 % (v/v) triton-X100, 0.5 % (w/v) RNase, and 0.005% (w/v) PI. Comparing to the control (without metal ions), Co^{2+} at 0.2 mM increased the percentage of the cells in gap2-mitosis, suggesting that Co^{2+} inhibited cellular function relating to mitosis. Mn^{2+} at 0.4 mM increased the percentage of the cells in gap0-gap1, suggesting that the cell cycle stopped within gap0-gap1. Hg^{2+} at 0.1 mM increased the percentage of the cell in DNA synthesis. V^{3+} at 0.5 mM increased the percentages of the cells both in DNA synthesis and in gap2-mitosis, which is the most popular case among 42 metal ions tested. Metal ions with relatively high cytotoxicity tend to increase the percentages of the cells gap2-mitosis. The data obtained will contribute to the elucidation of the mechanism of the cytotoxicity induced by metal ions.

THE CELLULAR RESPONSE TO CHROMIUM(VI) COMPOUNDS — THE DECISION BETWEEN LIFE AND DEATH

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Background: Environmental and occupational exposure to Cr (VI) constitutes a significant health risk. The contamination of drinking water with Cr (VI) compounds leads to the accumulation of chromium in the brain and liver of rats. Depending on exposure conditions, Cr (VI) may act either as an apoptosis inducer or a tumor promoter. However, the precise mechanisms of Cr (VI) toxicity have not been elucidated yet. Cr (VI) affects the expression of several redox-sensitive genes and causes activation of several transcription factors, suggesting the involvement of reactive oxygen species (ROS) whose generation accompanies the intracellular reduction of Cr (VI).

Aims: In an attempt to define mechanisms leading to apoptosis or carcinogenesis, the effects of different Cr(VI) insults on PC12, a good model for neuronal cells were investigated. After determining Cr(VI) exposures leading to apoptosis, cells were exposed to either apoptosis- or milder, non-apoptosis-inducing insults and the effects of these two kinds of insult on several oxidative stress parameters were determined.

Methods: Cell viability and duplication times were determined using MTT, apoptosis using the fluorescent probes Syto 13 and propidium iodide, ROS and Cr(V) formation using dichlorofluorescein, peroxidation extent using the thiobarbituric method, and carbonyl content and ATP levels using HPLC.

Results: When cells were exposed to 2–50 μM Cr(VI) for periods ranging from 2 to 24 h, a time- and concentration-dependent decrease in cell viability was observed. This decrease in cell viability was accompanied by similar time- and concentration-dependent decreases in ATP levels and increases in the peroxidation extent of the cells, in ROS levels and in Cr(V) formation

Conclusions: Cr(VI)-induced effects on PC12 cells are closely associated with a perturbation on the cellular redox status and the energy charge of these cells, implicating oxidative stress and mitochondria in the basal mechanisms underlying these effects. We are currently investigating the effects of 2 μM exposures, which did not induce a significant decrease in cell viability, on the growth rates of these cells.

SYNTHESIS, CHARACTERIZATION AND BIOACTIVITY OF POLYOXOMETALATES ON OSTEOBLASTS IN CULTURE

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Heteropoly- and isopolyoxometalates of V, Mo and W, represent a class of polyanionic compounds with a variety of important biological activities such as the inhibition of specific enzymes, antiviral and antitumoral effects. The K salt of the $[(PW_9O_{34})_2Co_4(H_2O)_2]^{10-}$ anion (I) has been obtained in aqueous solution from the precursor, $Na_8HPW_9O_{34}.24H_2O$ (II), and $CoCl_2$ in excess of KCl. Phases (I) and (II) were characterized by different techniques such as X ray diffraction (XRD), Vibrational spectrocopy (FTIR-Raman) and diffuse re-

flectance spectroscopy (DRS), scanning electron microscopy (SEM-EDAX) and thermal studies. Phase (I) is structurally related to the condensation of two fragments of (II) by a Co tetranuclear cluster in a sandwich type configuration. The biological activity on cell proliferation and morphological transformations, were investigated on rat osteosarcoma (UMR106) and mouse calvaria (MC3T3E1) — osteoblast-like cells in culture. Compound (II) did not show any effect on tumoral UMR106 proliferation while it induced the proliferation of MC3T3E1 cells in a bell like shape. On the contrary, phase (I) caused a strong inhibition on the UMR106 proliferation in a dose response manner. The latter observation correlated with the marked morphological changes caused by this compound on tumoral cells. In conclusion, compound (II) promoted the proliferation of non- transformed osteoblasts without any alteration of their morphology. This compound did not affect tumoral osteoblasts. On the contrary, phase (I) strongly inhibited tumoral osteoblast proliferation, inducing important morphological alterations. Hence, this compound is interesting from a pharmaceutical point of view for its potential use as an antitumoral drug.

IN VITRO EVALUATION OF BIOCOMPATIBILITY OF DENTAL METAL MATERIALS ON OSTEOBLAST CELLS IN CULTURE

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In clinical practice, different metallic biomaterials are used in prosthesis and orthodontic applications. Massive enrichment of metallic ions has been observed in the proximity of some biomaterials. Thus it is important to evaluate their biocompatibility with the adjacent tissues by using a reproducible system such as an in vitro bone-related cell culture. The aim of this work was to evaluate the biocompatibility of dental metal materials on the growth of osteoblast UMR106 cells in culture. Cells were grown in Dulbeccoґs modified Eagles media plus 0.5% fetal bovine serum with or without metal samples, for different incubation periods. Ag, Cu, Au, Pt, Pd and Ni/Ti alloy samples were assayed. Number of surviving cells, mitotic index, cell morphology, and the expression of alkaline phosphatase activity as a marker of osteoblastic phenotype were investigated as parameters of biocompatibility. The metal ion content of the culture media was analyzed using atomic absorption spectrophotometry. The results showed that Cu and Ag were the more cytotoxic elements, while Au, Pd, Pt and the Ni/Ti alloy were biocompatible in the osteoblastic culture. Cu and Ag induced strong morphological changes in the UMR106 cells after 24 hours of incubation. Cells became smaller, with loss of processes, condensation of cytoplasm, and the culture showed absence of mitotic figures. After 48 hours, only a few cell survived ($< 5\%$). Cells growing in the presence of Au, Pt, Pd and Ni/Ti survived well maintaining proliferation and osteoblastic differentiation similar to those of the control culture. These results were correlated with the release of metal ions in the media. In conclusion, our results showed that the UMR106 cell culture system appears suitable for evaluating the biocompatibility of metallic dental materials.

ERYTHOCYTE MAGNESIUM FLUX IN MICE SELECTED FOR HIGH AND LOWER ERYTHROCYTE MAGNESIUM LEVELS

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Intracellular Mg concentration is maintained at a physiological level preventing the accumulation of Mg in the cell or massive loss of this cation from the cell. Nutritional and genetic factors are involved in this cellular Mg homeostasis. The aim of this work was to explore cellular Mg flux in mice selected for high (MGH) and low (MGL) erythrocyte magnesium levels. Firstly, whole blood from the two mice selections was incubated for 2h at 37°C in the presence of ^{25}Mg. ^{25}Mg retention by erythrocyte was then determined. We observed lower retention of Mg in MGL mice erythrocytes after in vitro loading with ^{25}Mg by comparison to MGH. In fact, lower enrichments of ^{25}Mg in erythrocytes from MGL mice were observed ($39\% \pm 4$ and $49\% \pm 5$ for MGL and MGH respectively, $p<0.01$). In another study, we evaluated the efflux of Mg from Mg loaded erythrocytes. We demonstrated that this lower retention was partly due to higher Mg efflux in MGL erythrocytes. In fact, Mg efflux was 213 ± 31 mg/L cells/30 min for MGL and 171 ± 18 mg/L cells/30 min for MGH. Moreover, intracellular ATP,

the most important ligand of intracellular free Mg, was lower in MGL (53.4 ± 4.4 μmol/dl) by comparison to MGH (65.2 ± 5.2 μmol/dl) ($p<0.05$). These results reflect differences in Mg retention by the erythrocytes between MGL and MGH. Thus, a genetic control of Mg homeostasis seems to be involved in these cells.

VANADIUM INHIBITS HACAT CELL PROLIFERATION BUT DOES NOT CAUSE APOPTOSIS

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Vanadium is a transition metal widely distributed in the enviroment. Vanadium compounds exert a variety of biological responses including antiproliferative responses through the activation of signalling pathways and ROS. As the human skin keratinocyte is a cell type most exposed to enviromental insults, a spontaneously immortalised human keratinocyte cell line (HaCaT) bearing mutant, transcriptionally inactive p53, was used to investigate the antiproliferative effects of vanadyl (IV) sulphate on their growth behaviour. Treatment of HaCaT cells with 25 to 200 mM $VOSO_4$ for 24 h induced inhibition of cell proliferation in a dose-dependent manner, reaching a saturation level at 100 mM vanadate, as determined by colony formation assays. Growth inhibition was linked to suppression of serum-induced expression of *c-fos* proto-oncogene, an early response gene involved in skin homeostasis and induction of the Cdk inhibitor $p15^{INKB}$. Further, there was a slight induction of *clusterin/apolipoproteinJ*, a gene expressed by surviving cells under stressful conditions, following treatment with vanadate. Constitutive expression of *clusterin/apolipoproteinJ* in HaCaT cells failed to rescue them from vanadium-induced growth inhibition or to alter the pattern of expression of c-fos mRNA. We are currently investigating the effects of vanadium compounds and the role of clusterin/apolipoproteinJ in vanadium- and H_2O_2-induced antiproliferative responses.

CHROMIUM (VI) INTERFERANCE WITH MITOCHONDRIAL BIOENERGETICS

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Background: chromium (VI), an environmental contaminant, is widely recognized to exert toxic effects towards humans, animals and plants. Although the mechanism of Cr (VI)-induced toxicity is not clear, it is believed that the oxidative stress plays an important role. Mitochondria provide most of the cellular energy (ATP) and yield many intermediate compounds involved in normal cellular metabolism. Therefore, perturbations of mitochondrial function may result in severe consequences for general metabolism and all the energy transducing processes that require ATP. The strong decrease on the ATP levels detected in animal cells exposed to Cr(VI) has been closely correlated with the inhibition of mitochondrial respiration. In addition, daily oral low dose administration of Cr(VI) to rats results in enhanced lipid peroxidation in liver and brain mitochondria.

Aim: to clarify the mechanism of Cr(VI)-induced toxicity, the time and concentration-dependent effects of Cr(VI) on mitochondrial bioenergetics and membrane damage of mitochondria isolated from vegetal (turnip root) and animal (rat liver) sources were investigated.

Methods: mitochondrial bioenergetics was appraised by oxygen consumption, enzymatic activity of the respiratory complexes, mitochondrial transmembrane potential ($\Delta\Psi$) and mitochondrial membrane permeabilization to H^+ and K^+. Mitochondrial membrane damage by lipid peroxidation was examined by the assay of thiobarbituric acid reactive substances.

Results: chromium(VI) interferance with mitochondrial bioenergeticswas shown to decrease of the succinate-dependent respiratory indexes, (RCR and ADP/O); inhibition of oxygen-supported respiration by succinate or malate in state 3 and uncoupled respiration with parallel $\Delta\Psi$ dissipation and decrease of the phosphorylation efficiency; inhibition of the NADH-ubiquinone oxidoreductase (complex I) and succinate-dehydrogenase (complex II); alterations on the mitochondrial ATPase activity (complex V); and membrane permeabilization to K^+. The ubiquinol-cytochrome c reductase (complex III) and membrane permeabilization to H^+ were insensitive to Cr(VI). However, differences on the sensitivity to Cr(VI), $\Delta\Psi$ and phosphorylation efficiency, state 4 respiration and ATPase activities, and K^+ influx were detected between plant and animal mitochondria.

Conclusion: chromium(VI) interfers with plant and

animal mitochondrial bioenergetics as a function of concentration and exposition time, independently of mitochondrial membane damage induced by oxidative stress, but important differences were detected for the Cr VI) effects in plant and animal mitochondria. Chromium (VI)-induced perturbations on mitochondrial energetics will compromise energy-dependent biochemical processes, and, therefore, may contribute to the basal mechanism underlying its toxic effects in plant and animal cells.

EFFECT OF CADMIUM (II) AND LEAD (II) ON THE RADIATION ADAPTIVE RESPONSE VALUE IN RAT BONE MARROW CELLS

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Background: Heavy metals are the most widespread and harmful contaminants of environment. Cadmium and plumbum compounds have a particularly high genotoxic action. Normal cells are also capable of forming adaptive response (AR), an enhancement of cellular resistance to high challenge doses of ionizing radiation by preliminary exposure to low priming doses. Along with the wide distribution of metals in environment, prolonged chronic exposure to ionizing radiation at low dose rates often takes place.

The aim of the present work was to study the effect lead and cadmium, on the ability of animals to form AR in bone marrow cells of rats exposed to acute and chronic g-irradiation.

Materials and methods: Adult male rats (120-200 g) were exposed to chronic g-irradiation (^{137}Cs, dose rate of 0.13 cGy/h, cumulative doses were 3, 9, 21 and 40 cGy). Solutions of heavy metals Pb $(CH_3COO)2x3H_2O$ and $CdCl_2x2.5H_2O$ at a rate of 50 mg of pure substance per 1 l of boiled water were given to animals as a drinking throughout the period of chronic irradiation. Rats subjected to chronic irradiation with an appropriate dose were immediately subjected to single acute dose of 4 or 6 Gy (dose rate of 47 cGy/min). Chromosome preparations of bone marrow cells were carried out by standard procedure (Devi and Sharma, 1990). Percent of cells with aberrations and all types of chromosome aberrations were recorded.

Results: It was shown that treatment with Cd (II) and Pb (II) led to increase in frequency of chromosome aberrations in bone marrow cells of non-irradiated rats and slightly enhanced the radiation effect. Exposure to chronic γ-irradiation induced significant AR to subsequent acute irradiation. In the presence of Cd (II) and Pb (II) the magnitude of the AR was decreased 1.5- and 2-fold, respectively.

Conclusions: The administration of heavy metal salts to the diet of rats decreases the cytogenetic AR induced by chronic γ-irradiation.