1657 DMPS-MERCURY CHALLENGE TEST: URINARY MERCURY AFTER EXPOSURE TO MERCUROUS CHLORIDE

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The DIMAVAL (DMPS) challenge test (300 mg, po after an 11 hr fast) was given to 8 users of a skin lotion that contains mercurous chloride, 11 factory workers who make the skin lotion and 9 controls who had no known occupational exposure to Hg in Tampico, Mexico. Urines were analyzed for total Hg using cold vapor AAS. The μg Hg excreted for 6 hrs before and 6 hrs after DMPS treatment was 16.2 ± 3.43 and 1410 ± 346 for skin lotion users (n = 8); 113 ± 26 and 5037 ± 682 for factory workers (n = 11); and 1.08 ± 0.61 and 28.6 ± 12.1 SE for controls (n = 9), respectively. The increases in urinary Hg resulting from the DMPS challenge test were 87, 45 and 26-fold, respectively. Urinary coproporphyrin, precoproporphyrin and pentacarboxylporphyrin levels in all 3 groups decreased after DMPS administration. Coproporphyrin concentration before DMPS challenge was strongly correlated with urinary Hg excretion after DMPS challenge for the factory workers (r = 0.73) and the lotion users (r = 0.83). The results show the value of DMPS in humans for increasing urinary excretion of Hg and the value of the DMPS/MERCURY challenge test and urinary porphyrins for determining mobilizable Hg. (Supported in part by the Superfund Basic Research Program NIEHS Grant #ES-04940.)

1658 HUMAN AND RABBIT RENAL CORTICAL SLICES IN AERATED SUBMERSION CULTURE AS A MODEL TO EVALUATE HEAVY METAL CHELATORS

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Heavy metal chelators have been used for many years as therapeutic agents against metal poisonings by mercury, lead and arsenic. A submersion culture system has been developed to evaluate the efficacy of heavy metal chelators in removing metals from renal tissue. Slices were exposed to non-toxic loading doses of $HgCl_2$ (10 uM) and $NaAsO_2$ (50 uM) for 4 hrs prior to being transferred to the media (DME/F-12) containing the chelators 2,3-dimercapto-1-propane sulfonic acid (DMPS) or meso-dimercaptosuccinic acid (DMSA). DMPS and DMSA (0.5–10 mM) removed $NaAsO_2$ and $HgCl_2$ in a dose-dependent manner. Over 80% of the metals could be removed by 10 mM DMPS in 4 hrs of incubation. DMPS routinely removed more metal than DMSA. Even the lowest concentration of chelator (0.5 mM DMPS) removed 60% of the $HgCl_2$ from the renal slice, as compared to slices not exposed to chelators. The chelators were equally effective with either human or rabbit renal slices. The results of these experiments indicate that human and rabbit renal cortical slices are efficient models to evaluate heavy metal chelators as potential therapeutic agents. (NIEHS P42-ES-04940 and RO1-ES-05790).

1659 CHELATING AGENTS IN THE TREATMENT OF EXPERIMENTAL MANGANESE INTOXICATION

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The effects of seven structurally different metal chelators in the treatment of semichronic manganese (Mn) intoxication were assessed in male Swiss mice. Groups of 15 animals received daily s.c. injections of Mn (II) chloride tetrahydrate (32 mg/kg) for four weeks. Forty-eight hr after the last injection, chelation therapy was initiated and continued for 5 days. Mice received daily i.p. injections of 0.9% saline (control group), or cyclohexanediaminetetraacetic acid (CDTA), ethylenglycol-bis-(alfa-aminoethylether)-N,N'-tetraacetic acid (EGTA), N-(2-hydroxyethyl) ethylendiamine triacetic acid (HEDTA), isonicotinic acid hydrazine (INH), L-dopa, sodium 4,5-dihydroxy-1,3-benzenedisulfonate (Tiron) and p-aminosalicylic acid (PAS) at doses approximately equal to 1/8 of their respective LD_{50} values. Animals were housed in metabolic cages, and urine and feces were daily collected for 5 days. After this period, the animals were killed and tissues removed. Although none of the chelators increased the fecal Mn excretion, CDTA, EGTA, and HEDTA significantly enhanced the excretion of Mn into urine. Tiron, INH, and EGTA had no beneficial effect on tissue Mn concentrations, whereas PAS, HEDTA, and L-Dopa were only partially effective in reducing the tissue levels of Mn. In contrast, CDTA caused a significant decrease in the concentration of Mn in bone, brain, kidney and liver, the main target tissues of Mn accumulation. CDTA was the most effective chelator of those studied in the removal of Mn in Mn-loaded mice.

1660 RESPONSES OF LACTATING COWS TO MESO-2-3-DI-MERCAPTO SUCCINIC ACID (DMSA) USED TO INCREASE CLEARANCE OF HEAVY METALS

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DMSA has shown promise as a chelator to reduce burdens of metals in humans. Neither efficacy nor safety of this therapeutic agent has been demonstrated for cattle. Four lactating Jersey cows (521 ± 12 kg) were given 2 iv doses at 24 hr intervals of 0, 5, 15, or 45 mg/kg BW of DMSA in 400 ml of 5% $NaHCO^3$. Blood, urine, and milk were collected to determine changes in heavy metals. Unanticipated clinical signs of toxicosis, anorexia and depression, were evident within 24 hr of the first 15 and 45 mg/kg DMSA doses. For the 0, 5, 15, and 45 mg/kg treatments, urine production decreased by 22, 47, 74, and 85%, respectively, during the second 24 hr. By 72 hr, daily milk production decreased 18, 45, 100, and 100% from the mean of the 7 day predose period. Peak blood creatinine/BUN (mg/dl) was 0.8/9.0, 1.0/11.0, 15.7/192.0, and 10.2/127.0, respectively. The 15 mg/kg treated animal was moribund 9 days after the first dose. Necropsy revealed severe acute renal tubular necrosis with protein and cellular casts. The cow dosed with 45 mg/kg was recovering by day 15, with blood creatinine and BUN approaching normal and limited production of milk returning. Kidneys of cattle appear to be a target organ for DMSA and the threshold of toxicity should be determined.

1661 ROLE OF CALCIUM AND PHOSPHOLIPASE IN POTASSIUM ANTIMONYL TARTRATE-INDUCED CARDIAC MYOCYTE TOXICITY

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Exposure of rat cardiac myocytes to potassium antimonyl tartrate (PAT) has been shown to cause oxidative stress and toxicity. This study investigates the roles of intracellular calcium ($Ca^{2+}i$) and phospholipase (PLPase) activation in PAT-induced toxicity. Myocytes isolated from rat neonates were cultured on plastic culture dishes or glass coverslips and were used after 2–3 days in culture. Toxicity and lipid peroxidation were assessed by release of lactate dehydrogenase (LDH) and thiobarbituric acid reactive substances (TBARS), respectively. $Ca^{2+}i$ was monitored on an inverted phase contrast microscope with the fluorescent probe fura-2. ATP content was measured by extraction and HPLC analysis. Exposure to 200 μM PAT for 4 hrs resulted in a 50–60% release of LDH (% of total) and a 10-fold increase in TBARS release. In individual fura-2 loaded myocytes, 200 μM PAT increased $Ca^{2+}i$ by approximately 75% by 3 hours, whereas, exposure to 500 μM PAT resulted in $Ca^{2+}i$ overload (greater than 10-fold increase). Preloading of myocytes with the Ca^{2+} chelator BAPTA (10 μM) prevented the effects of PAT on LDH and TBARS release, but PAT exposure in calcium-free/EGTA medium did not prevent toxicity, suggesting release from intracellular Ca^{2+} stores. Co-exposure to the PLPase inhibitor mepacrine also prevented PAT toxicity. After 6 hrs exposure to PAT (200 μM) in the presence of mepacrine (50 μM) LDH release was still prevented, but myocytes were hypercontracted, suggesting an elevated $Ca^{2+}i$. PAT-induced ATP loss was partially prevented by BAPTA and mepacrine. These results link elevated $Ca^{2+}i$ and activation of PLPase with PAT-induced myocyte cell death and suggest that increased $Ca^{2+}i$ precedes phospholipase activation.

1662 EFFECTS OF ANTIMONY ON MITOCHONDRIAL FUNCTION AND PROTEIN THIOL AND ADENINE NUCLEOTIDE STATUS IN CULTURED CARDIAC MYOCYTES

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Antimony and antimony containing compounds are used in the manufacture of paints, pigments, ceramics, pyrotechnics, fire retardants and glass. Exposure to antimony containing compounds has been associated with cardiac toxicity in man and experimental animals. In a previous study in our laboratory, we demonstrated that potassium antimonyl tartrate (PAT) depletes cellular glutathione and induces oxidative stress and toxicity in rat cardiac myocytes. In the present study, cardiac myocytes isolated from neonatal rats and cultured for two days were used to further examine the mechanism of PAT-induced

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