1635 RESTRAINT STRESS AND OTHER NEUROPROTECTIVE MANIPULATIONS INCREASE BRAIN CONCENTRATION OF d-3,4-METHYLENEDIOXYMETHAMPHETAMINE (D-MDMA) IN C57BL/6J MOUSE.

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Temperature lowering manipulations, including restraint stress as well as co-administration of temperature lowering agents have proven protective against the dopaminergic neurotoxicity induced by repeated doses of d-MDMA. It is reasonable to expect that such neuroprotective effects may arise from an alteration in the amount of toxic compound that is able to reach the target site, in this case the striatum of mouse brain. Therefore, we determined brain concentrations of unchanged drug at two hours after completion of drug administration (d-MDMA 15 mg/kg, s.c. given every 2 hours for a total of 4 doses) using an HPLC assay with fluorimetric detection of d-MDMA. Restraint stress increased by 3 fold the amount of d-MDMA that reached striatum. Similarly, co-administration of ethanol 3 gm/kg (s.c. given 30 minutes before the first and third doses of d-MDMA) increased by 5 fold the striatal concentration of d-MDMA. Both these manipulations are associated with neuroprotection. The results imply that prevention of the neurotoxic effects of d-MDMA is due to altered pharmacokinetics. The results suggest that dopaminergic neurotoxicity induced by d-MDMA is a result of drug metabolism which yields a product that is toxic to nigrostriatal neurons of mouse. Both restraint stress and ethanol co-administration may be neuroprotective by inhibition of the production of a toxic metabolite of d-MDMA. An equally neuroprotective manipulation, co-administration of MK-801 1mg/kg s.c. 30 minutes before the first and third dose of d-MDMA, reduced body temperature but only doubled the concentration of d-MDMA in striatum. All these manipulations lower body temperature but alter distribution to disimilar extents. Overall these data suggest that there may be multiple mechanisms of neuroprotection against d-MDMA-induced dopaminergic neurotoxicity in mouse. Supported by CDC-NIOSH.

1636 ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION (ADME) STUDIES OF ^{14}C-EMTRICITABINE (COVIRACIL®; FTC) IN MONKEYS AND RATS.

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FTC is a nucleoside analog which is a potent reverse transcriptase inhibitor in Phase III clinical development for the treatment of HIV and HBV infections. ADME studies of ^{14}C-FTC were done in male cynomolgus monkeys (CM; n=4), and in male Sprague-Dawley (SD; n=3/time point) and Long-Evans (LE; n=1/time point) rats. A single oral dose of 200 mg/kg containing 50 µCi/kg ^{14}C-FTC was given to CM. The same dose was given 3 weeks later and at 1 hr, the CM were euthanized and blood and tissues collected. Rats were given a single oral dose of 200 mg/kg containing 135 µCi/kg ^{14}C-FTC. Tissue distribution of ^{14}C radioactivity (RA) was studied using quantitative whole body autoradiography. Urine, feces and blood were collected at intervals up to 120 hr (CM) and 144 hr (rats). All samples were analyzed for total RA by liquid scintillation counting. Metabolites were studied using HPLC with radiometric detection (CM and SD) coupled with LC/MS/MS (CM; in progress). In CM, RA peaked at 1 hr in blood/plasma, with detectable levels up to 48 hr post dose. Excluding the GI tract, the highest RA levels were found in the kidneys and liver. Mean total dose recovery was 41% in urine and 35% in feces (8% in cage wash). RA distributed well into aqueous humor, brain, CSF and bone. In SD, distribution of RA to tissues was rapid, with C_{max} occurring at 1 hr. Levels then declined rapidly and were undetectable in all tissues studied at 24 hr. The highest levels were found in (excluding GI tract) the kidneys, liver and bladder. Terminal half-lives of RA ranged from 1.2 hr (kidneys and liver) to 2.7 hr (21 other tissues). Distribution of RA was similar in SD (non-pigmented) and LE (pigmented) rat tissues, indicating there was no significant binding of FTC to melanin. In SD, >95% the RA in 0-24 hr urine and feces samples existed as parent drug, respectively. In both species, FTC was rapidly absorbed following oral administration, widely distributed to all tissues examined, and rapidly excreted, primarily as parent drug.

1637 NEGLIGIBLE DEPLETION SOLID PHASE MICROEXTRACTION (ND-SPME) IN STUDYING PROTEIN BINDING.

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For studying protein binding of compounds, the assays applied usually (*e.g.*, saturation binding assays) measure the bound fraction of the compound. Saturation binding assays assume that the free concentration is identical to the nominal concentration, but for hydrophobic chemicals, this is hardly the case. Measuring the free fraction is then inevitable. In general, information on the freely dissolved fraction of a chemical *in vitro* and in vivo is essential because this fraction is responsible for the activity of a compound. Until now, measuring the free fraction in protein binding assays has been possible only with equilibrium dialysis. This is a time-consuming method, where loss of compound due to binding to the membrane can occur. Solid phase microextraction (SPME) has been shown to be a suitable and simple method for measuring freely dissolved concentrations if it is performed in a negligible depletion manner. To determine the association constant (K_a) of a compound from the free fraction (ff), one can use Rowland and Tozer's relation: $ff = 1/(1 + K_a * f_p * P_t)$; where f_p is the unoccupied fraction of protein and P_t is the total protein concentration. When the protein concentration is much larger than the compound concentration, then $f_p \approx 1$ and K_a can simply be determined by measuring the free fraction with different protein concentrations. Also with competitive binding assays, K_a can be determined from the free concentration of a radioligand, by determining the relative binding affinity (RBA): $RBA = IC_{50,reference}/IC_{50,competitor}$ $K_{a,competitor} = K_{a,radioligand}/[(1/RBA)(1 + R) - R]$ In this equation R is the bound to free ratio of the radioligand at 50% displacement. This new method for studying protein binding is currently being validated in binding studies to bovine serum albumin (BSA) of estradiol and series of hydrophobic organic chemicals. Protein binding affinities are determined and the results will be compared with those of a classical method.

1638 THE EFFECT OF PREGNANCY ON RENAL CLEARANCE OF BORON IN HUMANS: A STUDY BASED ON NORMAL DIETARY INTAKE OF BORON.

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Humans consume about a milligram of boron daily, mostly from fruit and vegetables. At high doses, boron is a developmental and reproductive toxin in animals, and pregnant rats were the most sensitive species. To extrapolate from the large, animal boron toxicity database to humans, especially pregnant women, information on renal clearance of boron was needed. Therefore, we measured renal clearance of boron in pregnant (P) and non-pregnant (NP) women. In 16 second-trimester women with a mean age of 26.8 yrs and 15 NP age-matched referents with a mean age of 26.7 yrs, dietary boron intake, blood and urine boron concentrations, and renal boron clearance were determined. Boron intake in P and NP groups was 1.35±0.64 and 1.31±0.47 mg boron/24 hrs respectively. Blood and urine for boron, creatinine and urea was collected at the start, at 2 hrs in the Clinical Research Center, and at 24 hrs. Plasma boron concentrations were 0.023±0.015, 0.018±0.011, and 0.013±0.006 ug/ml in P, and 0.022±0.013, 0.024±0.015, 0.027±0.018 ug/ml in NP at baseline, 2 and 24 hrs repectively. Renal boron clearance measured over the initial 2 hrs, the most complete urine collection period, was 68.30±35.00 ml/min/1.73 m2 for P and 54.31±19.35 ml/min/1.73 m2 for NP subjects. Fractional excretion of boron obtained by determining the ratio of boron to creatinine clearance (57.3±32.3% in P and 46.7±13.6% in NP at 2 hrs) demonstrated that tubular reabsorption of boron occurred in both P and NP women.

1639 96-WELL PLATE SCREENING ASSAY FOR EVALUATION OF POTENTIAL HEPATOTOXICITY OF XENOBIOTIC COMPOUNDS.

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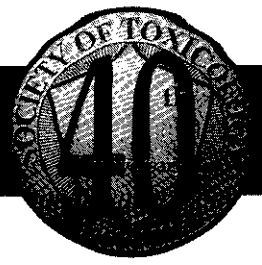
Determination of the potentially toxic effects of drug candidates remains a crucial step in drug discovery. In order to evaluate the hepatotoxic effect of drugs, we designed a rapid screening assay. Initially, rat hepatocytes were isolated by a two-step collagenase perfusion and cultured under different conditions for optimization of the assay. The 96-well plate format allowed us to screen eight compounds at five concentrations up to 1 mM per plate. Cytotoxicity, as indicated by the extend of cell injury, was measured by lactate dehydrogenase (LDH) leakage into the culture medium and expressed as a percentage of total LDH activity (% LDH total). Following assay optimization, monolayers of hepatocytes cultured on a collagen-coated dish were maintained for 24 hours in presence or absence of xenobiotics for hepatotoxicity assessment. EC_{50} values were determined graphically from a semi-logarithmic plot (GraphPad Prism Software), using "Real LDH values (% LDH leakage in presence – absence of the compound)" and represented the concentration causing a 50% increase of LDH leakage over this period. Stock solutions (10 X) of reference compounds (excluding $CdCl_2$) were prepared using DMSO (10%),



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Preface

This issue of *The Toxicologist* is devoted to the abstracts of the presentations for the symposium, platform, poster discussion, workshop, roundtable, and poster sessions of the 40th Annual Meeting of the Society of Toxicology, held at the Moscone Convention Center, San Francisco, California, March 25–29, 2001.

An alphabetical Author Index, cross referencing the corresponding abstract number(s), begins on page 451.

The issue also contains a Keyword Index (by subject or chemical) of all the presentations, beginning on page 479.

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