● 404 THE CLINICAL PHARMACOLOGY OF IMIPENEM (IMP) AND CILISTATIN (CIL) IN PREMATURE INFANTS.

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IMP/CIL is a new combination ß-lactam antibiotic which possesses potent in vitro activity against a broad range of pathogens commonly isolated from premature (P) and newborn infants. To assess dosing requirements, P were assigned to 10,15,20 or 25 mg/kg IMP/CIL intravenously over 15 to 40 min either as single or multiple doses. 30 P (670-1890 gm) from 24.5-36 weeks gestation were studied. All were studied during the first dose (FD); 12 were restudied after at least 10 doses (SS) given q12h. All studies were performed during the first week of life. Multiple blood and urine samples (over 8-12 hrs) were obtained for the determination of IMP/CIL by HPLC. Pharmacokinetic (PK) analysis was performed using standard non-compartmental methods. Total (n=29) FD PK revealed mean (±SD) IMP: t1/2=2.4(0.3), Vdss=0.5(0.1) L/kg, Cl=47(12) ml/min/1.73m^2 and CIL: t1/2=8(2.8), Vdss=0.4(0.1)L/kg, and Cl=10.5(3.5) ml/min/1.73m^2. IMP/CIL renal Cl averaged 8.7(4.2) and 4.0(2.5) ml/min/1.73m^2, respectively. No differences were observed in PK parameters (except AUC) for the different doses studied or between IMP FD and SS evaluations. In contrast, SS CIL AUC and t1/2 were decreased and body Cl increased from FD ($p<0.02$). IMP/CIL AUC correlated directly with dose administered: FD IMP r=0.74 and CIL r=0.64. Peak IMP/CIL concentrations averaged 23.6 and 32.1 mg/L after 10 mg/kg FD increasing linearly over the dose range studied. A direct relationship between post conceptual age (PCA) and FD IMP/CIL body Cl (r=0.61/0.55) and renal Cl (r=0.31/0.68) was observed. No drug related toxicities were observed in any P. Depending on PCA, doses should range from 15 to 25 mg/kg administered q12h to maintain therapeutic peak and trough IMP serum concentrations. CIL body Cl increases rapidly and to a greater extent than IMP during the first week of life.

405 SULFAMETHOXAZOLE HYPERSENSITIVITY REACTIONS ARE MEDIATED BY A HYDROXYLAMINE METABOLITE

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The pathogenesis of idiosyncratic "hypersensitivity" reactions to the sulphonamides is thought to be mediated by reactive intermediates. We have previously demonstrated dose-related toxicity of reactive intermediates of sulphonamides generated by murine microsomes using an *in vitro* lymphocyte assay. We have previously described the production of sulphonamide hydroxylamine by murine microsomes, and have synthesized pure hydroxylamine sulfadiazine and sulfamethoxazole. The toxicity of the hydroxylamine derivative of sulfamethoxazole was tested in ymphocytes of 11 normal volunteers, 3 patients who had sustained idiosyncratic reactions to the sulphonamides and a patient with GSH-S deficiency Cells from the patients had been tested with our microsomal lymphocyte assay and found to be sensitive to sulphonamides in the presence of a metabolic activating system. Hydroxylamine toxicity was assesed by the ability of the cells to convert MTT (tetrazolium) to a purple formazan using an automated microtitre plate assay. Toxicity of the hydroxylamine was greater in cells from patients who had hypersensitivity reactions to the sulphonamides. At 25 µg/ml, the control cells had 47.9% cell death (95% confidence limit 39.7 - 56.2), compared to the patients toxicity of 65.3 cell death (58.9 - 71.7). Similarly, at 50 µg/ml the control toxicity was 58.6% cell death (47.1 - 70.1) compared to the patients toxicity of 82.6% cell death (78 - 87.3). GSH-S deficient cells displayed a marked increase in dose-related toxicity throughout the concentration range. These results support the theory that idiosyncratic reactions to the sulphonamides are the result of abnormal detoxification of hydroxylamine metabolites, perhaps by glutathione-mediated pathways.

● 406 CHEMICAL SYNTHESIS AND *IN VITRO* TOXICITY OF A REACTIVE INTERMEDIATE OF SULFAMETHOXAZOLE

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Idiosyncratic "hypersensitivity" reactions to sulphonamides may be mediated by reactive intermediates. The diagnosis of these reactions is often difficult; moreover, the pathogenesis of these reactions has been extremely difficult to understand and study. We have demonstrated the cytochrome P-450 dependent production of hydroxylamine metabolites of the sulphonamides by murine microsomal preparations. In order to pursue the role of such a metabolite in mediating sulphonamide toxicity, we set out to synthesize and characterize the hydroxylamine (HA)derviative of sulfamethoxazole (SMX). Synthesis of the HA was initiated by mixing 4-nitrobenzene sulfonlyl chloride and 3-amino-5-methylisoxazole in pyridine. Nitro-sulfamethoxazole was recrystallised and dissolved in ethanol and reduced under hydrogen in the presence of a poisoned catalyst. The HA was recrystallized using a mixture of ethyl acetate and toluene. Analysis by TLC and HPLC demonstrated that the product was 95% pure. The HA was then used in a lymphocyte assay using cells from a normal volunteer . Toxicity was assessed by using MTT (tetrazolium) as a marker in an automated microtitre plate assay. Over a range of 0.1 to 10 mM, the HA produced concentration-dependent toxicity (39% deal cells at 10 mM/ml). SMX was non-toxic even at 10 mM. In the presence of murine hepatic microsomes, SMX produces toxicity. At 750 mM, cell death is comparable to 2.5 mM HA, suggesting a 0.3% conversion to the metabolite by microsomes. Chemically-synthesized reactive intermediates such as this compound are useful in studying the pathogenesis of poorly-understood adverse reactions such as idiosyncratic reactions attributed to the sulphonamides; additionally, these compounds may be very useful in the development of diagnostic tests that will quickly, accurately and safely diagnose these adverse reactions.

407 **A STUDY ON THE INTRAVENOUS TOXICITY OF E-FEROL IN THE NEONATAL RABBIT:**

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The intravenous administration of E-Ferol in premature infants has been associated with a fatal syndrome of ascites, splenomegaly, hepatomegaly, cholestatic jaundice, azotemia and thrombocytopenia. This study was undertaken to further delineate the intravenous toxicity of E-Ferol and its vehicle. Albino rabbit pups were delivered at 30 days gestation by c-section under halothane anesthesia. Central venous catheters were placed 2-6 hours after birth and parenteral nutrition was provided for 7 days. Four treatment groups were utilized 1) alpha-tocopherol (AT), 2) alpha-tocopheryl acetate (TA), 3) polysorbate (P) and 4) saline control. The vitamin E preparations were formulated by incorporating 25 mg of TA or AT per ml of vehicle solution comprised of 9% P 80 and 1% P 20. A dose of 4 ml/kg was administered daily. The weight gain of the animals over the 7 day period averaged 1.0 g, -0.1 g, -1.6 g and 3.0 g for groups 1,2,3 and 4 respectively. AT concentration was significantly ($p<0.01$) greater in liver, lung and plasma of animals supplemented with vitamin E. Liver vitamin E levels in supplemented and unsupplemented animals were (mean±SD) 4340±2249 ug/g and 65±25 ug/g respectively while plasma levels were 99±72 ug/ml in supplemented animals and 16±23 ug/ml in unsupplemented animals. There was a trend for higher bilirubin concentrations and Alk Phos activity in group 1,2 and 3 as compared to saline control. There were no differences in BUN or creatinine concentrations as well as in ALT, and GGT activity amongst the groups. Conclusions: 1) Levels of vitamin E in liver, lung and plasma reflected the specific treatment group 2) Significant accumulation of vitamin E occurred in the liver 3) The limited number of animals studied coupled with a limited nutritional regimen precluded assignment of toxicity to vitamin E versus the polysorbate vehicle.

408 PHENYLISOPROPYL ADENOSINE (PIA) PREVENTS INCREASE IN PULMONARY ARTERY PRESSURE DURING HYPOXIA IN NEWBORN LAMBS.

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Adenosine analogs are potent vasodilators. In newborn lambs we tested the hypothesis that PIA would decrease the pulmonary artery pressure (PAP) without significant decrease in aortic pressure (AoP). Catheters were placed in the pulmonary artery, aorta and inferior vena cava. After 48 hr. the following variables were measured:PAP, AoP, heart rate (HR), respiratory rate (RR), and arterial blood gases. Five sets of experiments were performed in 3 animals: 1) hypoxia (9% FiO2), 2) PIA, 3) PIA+hypoxia. A dose-response curve for PIA (5 to 60 mcg/kg IV bolus) was established. Mean PAP increased with hypoxia (from 12±3 to 27±2 torr $p<0.001$, PaO2 18±3 torr). After PIA pre-treatment, hypoxia (PaO2 24±3 torr) did not change the mean PAP (13±4 to 13±6 NS). The AoP did not change with hypoxia, nor with PIA + hypoxia (85±4 to 88±12 and 87±11 to 74±20 torr, NS). HR increased with hypoxia (from 165±21 to 320±39 $p<0.004$) and decreased with PIA (191±18 to 145±21/min., $p<0.007$). RR increased with hypoxia (38±7 to 56±8/min. $p<0.03$). PaO2 decreased during hypoxia and PIA+hypoxia (79±11 to 18±3, $p<0.008$, and 79±4 to 24±3 torr, $p<0.001$). PCO2 and pH did not change. These results suggest that PIA prevents increase in PAP during severe hypoxia without decreasing the AoP.

409 EFFECT OF VEHICLE AND ROUTE ON CLONAZEPAM LEVELS IN RATS.

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Clonazepam (CLZ) is a useful anticonvulsant; however, studies using different vehicles and routes have found conflicting results. CLZ levels measured in rats after single dose injections of either intraperitoneal (IP) or subcutaneous (SC) CLZ (1.0 mg/kg) dissolved in either propylene glycol (PG), propylene glycol + 18% ethanol (PGEtOH) or polyethylene glycol (PEG 400) were used to calculate the terminal elimination rate constants (beta), area under the time-concentration curve (AUC) and total clearance (Cl_T). A two way analysis of variance between route of administration and vehicle showed an effect of route on Cl_T ($P<0.001$) which depended on which vehicle was administered. When CLZ was dissolved in PG or PEG 400 the beta and Cl_T was significantly ($P<.05$) lower, and the AUC was significantly ($P<.05$) higher after SC than IP injections. The differences in beta, Cl_T or AUC between SC and IP injections were not significant when CLZ was dissolved in PGEtOH. SC injections produced more stable and reproducible CLZ levels than IP injections regardless the vehicle used. CLZ injections produce different plasma levels depending on the route and the vehicle used. In order to compare studies of CLZ effects, all of these variables must be considered.