

SECTION VI

**PHARMACOLOGY GRADUATE STUDENT-
RESIDENT RESEARCH REPORTS**

Chairperson
Dr. Peter Eyre

PHARMACOKINETICS, PHARMACODYNAMICS AND SUBACUTE ORAL TOXICITY OF CYCLOSPORINE-A (CyA) IN PIGS

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This investigation evaluated the pharmacokinetics, pharmacodynamics, and subacute oral toxicity of CyA in pigs. Nine 3-4 month old female Yorkshire pigs were given 10 mg/kg CyA IV. Four days later, the pigs were randomly divided into three groups (n=3) and given CyA per os (Gp A 12.5, Gp B 25, and Gp C 50 mg/kg). The resulting pharmacokinetic parameters were [mean (SD)]: A, 5.57 (2.53) ug/ml; α , 1.26 (0.89) hr⁻¹; B, 1.49 (0.84) ug/ml; B, 0.098 (0.03) hr⁻¹; T 1/2 α , 0.96 (0.7) hrs; T 1/2 β , 7.71 (2.6) hrs; VD_{ss}, 4.47 (2.22) l/kg; V_c, 1.71 (0.78) l/kg; Cl_B, 8.95 (2.7) ml/kg/min; oral bioavailability (F), overall 0.57 (0.19), Gp A 0.44 (0.11), Gp B 0.78 (0.15), Gp C 0.48 (0.06); time to peak concentration, 3.55 (0.88) hrs. Following the oral study, each pig was given the same oral dose as in the pharmacokinetic study, daily for 22 days. Whole blood 24 hour trough concentrations (ng/ml, RIA, monoclonal antibody) were as follows: Gp A, 224.3 (78.4); Gp B, 640.7 (174.6); Gp C 2344 (1095). Lymphoblast transformation stimulation indices were suppressed in all pigs except for one in Group A which had a corresponding CyA concentration of 92.4 ng/ml. One pig in Gp A had mild hepatic necrosis; one in Gp B had increased serum bilirubin concentrations. Pigs in Gp B and C developed mild renal tubular dilation. Significant decreases in serum albumin were detected in all groups; significant decreases in serum magnesium occurred in Gps A and B.

ELISA AND RIA DETECTION OF FENTANYL IN THE HORSE

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We have developed and evaluated an ¹²⁵I radioimmunoassay (RIA), and a one step enzyme-linked immunosorbent assay (ELISA) for fentanyl, a narcotic analgesic, in racing horses.

The ¹²⁵I-fentanyl RIA is highly sensitive, capable of detecting administration of doses that are 100 times less than

that required to produce a desirable pharmacological effect. Ten to twenty urine samples can be pooled and screened concurrently, pools flagged positive can be re-analyzed and the sample containing fentanyl can be isolated.

The ELISA test detects fentanyl with an I-50 of about 100 pg/ml. The antibody also cross-reacts with the methylated analogs of fentanyl. It is rapidly applicable on the field, and is capable of detecting these drugs and their metabolites in horse blood or urine. This test would likely improve the quality and reduce the cost of drug screening in horse racing.

This study was supported by a grant, "Immunoassay Tests for High Potency Narcotic Analgesics in Racing Horses" from the Kentucky Equine Drug Research Council and Kentucky State Racing and Harness Racing Commissions.

BINDING OF DOXYCYCLINE TO PLASMA ALBUMIN OF SEVERAL SPECIES

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Association constants (K_a) and number of binding sites (n) were determined by means of double reciprocal plots and Scatchard plots to characterize the interaction of doxycycline with plasma albumin of several species. Albumin fraction V (Sigma Chemical) was dissolved in 0.2 M NaPO₄H at a concentration of 2 X 10⁻⁴ M. Solutions of doxycycline in distilled water (free fraction) or acetonitrile:85% phosphoric acid:water (20:2:78; total fraction) were mixed 1:1 with the albumin preparations to obtain final doxycycline concentrations of 40, 50, 60, 70, 80, 90, 100, 200 and 300 ug/ml. Separation of the bound and free fractions was achieved by ultrafiltration using a microseparation system (Centricon 30, Amicon, Danvers, MA).

	K _a (liters/mole)	1/n
cat	9160	0.553
dog	3260	0.434
pig	3900	0.344
sheep	12370	0.542
cow	10860	0.880
man	2440	0.287

These results indicate differences in the *in vitro* affinity of doxycycline for albumins originating from several species. Because the extent of protein binding is known to influence the value of pharmacokinetic parameters, these findings may help to elucidate species-related dissimilarities in pharmacologic and toxic responses.

EFFECTS OF ILOPROST ON THE PHARMACOKINETICS AND RENAL DISPOSITION OF CYCLOSPORINE IN SHEEP

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Cyclosporine (CsA) is a nephrotoxic, cyclic undecapeptide currently used as an immunosuppressive drug. Iloprost (Il), a synthetic prostacyclin, may ameliorate the decrease in glomerular filtration rate (GFR) and renovasoconstriction caused by CsA, because Il dilates renal blood vessels. Six female sheep had both kidneys surgically translocated to a subcutaneous location posterior to the 13th rib. They were maintained for 5 months before the study, and renal function evaluated by serial BUN and serum creatinine and was stable. Ewes were divided into 2 equal groups; group 1 (G1) received only CsA at a dosage of 5 mg/kg as a three hour IV infusion once daily for 5 days (days -4 to 0 of the study). Group 2 (G2) received CsA similarly and was co-infused with Il at a dosage rate of 0.2 μ g/kg/min once daily from days -4 to 0. Serum and kidney samples (taken by percutaneous biopsy of the left kidney on days 1, 3, 6, 12, and 18) were taken for CsA analysis, GFR was determined by plasma radionuclide clearance on days -4 and 0 of the study, and plasma renin activity (PRA) was determined on days -4, -2, 0, 1, 6, 12, and 18. Necropsy and histopathologic examination of kidneys taken on day 18 indicated no differences between G1 and G2. Further, there was no difference in biopsied vs. nonbiopsied kidneys of all sheep. GFR increased from day -4 to day 1 in both G1 and G2 ($P < 0.05$), but there was no difference in GFR between G1 and G2. PRA in G2 increased during dosing (day -4 to day 0), but returned to baseline after day 0. PRA in G1 remained stable from day -4 to day 0, increased on day 1, then returned to baseline.

OPERANT CONDITIONING IN THE HORSE: A METHOD OF DETECTING SUBTLE CHANGES IN BEHAVIOR

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Variable interval (VI) reinforcement scheduling is a specific type of operant conditioning that is sensitive to drug effects even when overt clinical signs of the drug have dimi-

nished. We conditioned six horses to break a light beam with a head-bobbing movement and reinforced this behavior with a reward of clean oats (approximately 30 mg/ reinforcement). Initial training procedures included acclimation to the behavioral equipment and fixed-ratio reinforcement scheduling. To establish baseline rates of behavior the horses were converted to a variable interval (60 seconds) reinforcement schedule and were kept on this schedule for the remainder of the study. Daily sessions lasted 30 minutes per horse and their responding rates were remarkably stable even after long periods of inactivity. Responses and reinforcements were recorded and dispensed by use of an electromechanical relay system wired to an electric eye, an automatic feeder and a programming and recording system. Detomidine, hordenine, saline and xylazine were administered to the horses with responding rates monitored and compared to previous studies using cocaine and reserpine. Subtle changes in behavior induced by these agents were readily detected by use of this method.

DIFFERENTIAL EFFECTS OF MU AND KAPPA NARCOTIC AGONISTS IN THE HORSE

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The pharmacologic effects of selected μ - and kappa-receptor narcotic agonists were investigated in the horse. Fentanyl, a prototypic μ -receptor (morphine like) agonist and U-50,488H, a selective kappa-receptor (ketazocine) agonist were assessed for ability to produce analgesia against a noxious heat stimulus. In addition, changes in autonomic parameters of heart and respiratory rates, as well as locomotor stimulation were assessed after administration of fentanyl, U-50,488H and saline.

Both the μ -receptor agonist (fentanyl) and the kappa receptor agonist (U-50,488H) were effective in producing analgesia of variable duration against a noxious thermal stimulus. However, the kappa receptor agonist, U-50,488H, induced less change from resting state values on cardiac, respiratory and locomotor parameters when compared to saline controls than did the μ -receptor agonist, fentanyl. This experimental evidence suggests that selective kappa receptor narcotics may provide a useful group of equine analgesics without the undesired attributes of classic μ -receptor narcotics.

PHARMACOKINETICS OF VANCOMYCIN HYDROCHLORIDE IN THE HORSE FOLLOWING INTRAVENOUS INFUSION

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Vancomycin is used to treat severe staphylococcal and enterococcal infections in humans. Ototoxic and nephrotoxic side effects are potential problems if peak levels exceed 40 mg/L. The pharmacokinetics of vancomycin were determined for serum and synovia of healthy horses given single-dose IV infusions. Five healthy mature horses were used in the study. There were three groups of 5 animals in each group. These animals were each assigned to the 3 dosage schedules; 3.0 mg/lb (6.6 mg/kg); 5.0 mg/lb (11.0 mg/kg); and 7.0 mg/lb (15.4 mg/kg). Vancomycin hydrochloride solution, containing 500 mg per vial, was diluted to a volume of 10 ml and administered at the 3 dosage schedules described. The dosages were administered by intravenous infusion over a 30 minute period. Samples were collected from the jugular vein at 0.25, 0.5, 1, 2, 4, 6 and 12 hours after injection. Synovial fluid samples were collected at 0.5, 1, 2, 3, 4, 5, 6 and 8 hours postinjection through an aseptically placed catheter in the radial carpal joint. All samples were stored at -70° C and assayed at the same time. Minimum inhibitory concentrations (MIC), minimum bactericidal concentrations (MBC) were run on *Staphylococcal* and *Enterococcal spp.* isolates from the clinical microbiology service. The pharmacokinetics of vancomycin hydrochloride will be presented. A dosage regimen will be recommended based on the pharmacokinetic data and MIC/MBC's.

THE *IN VITRO* RESPONSIVENESS OF THE EQUINE DIGITAL ARTERY AND VEIN: EVIDENCE FOR THE ETIOLOGY OF LAMINITIS

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The effect of endothelial cell damage, endotoxin treatment and acute laminitis on normal pharmacological responsiveness of digital arteries and veins was studied.

Digital arteries and veins were removed from normal horses freshly killed at a local abattoir or from horses with experimentally-induced laminitis. Spirally cut strips of arteries and veins were suspended in isolated organ baths containing Krebs-Henseleit solution. Spasmogenic responses to phenylephrine and serotonin and spasmolytic responses to isoproterenol, ATP, bradykinin, prostacyclin (PGI₂) and acetylcholine were recorded using an isotonic myograph apparatus.

Spasmogenic responses to serotonin were significantly enhanced ($p < 0.05$) in digital arteries from laminitic animals. Vasodilatory responses to ATP were decreased ($p < 0.05$) in vessels from laminitic animals. Endotoxin treatment resulted in decreased responses to phenylephrine and isoproterenol in digital arteries ($p < 0.05$). Vessels denuded of endothelium showed a significantly decreased response to PGI₂ ($p < 0.05$).

The results of this study suggest that laminitis in horses could be the result of impaired vascular response which may be associated with endothelial damage and the effects of endotoxin.

SECRETION IN EQUINE AIRWAYS

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Increased airway secretion is associated with hyperresponsive allergic airway disease ("heaves") in horses. The mechanism of electrolyte secretion and the effects of inflammatory mediators have not been characterized in equine trachea. To understand the mechanism and regulation of secretion, equine trachea (stripped of serosal muscle) was mounted in Ussing-type chambers and bathed in Ringer solution with plasma-like ion concentrations. The tissue generates a serosal positive trans-epithelial potential (P.D.) of 22 mV and short circuit current (Isc) of -165 $\mu\text{A}/\text{cm}^2$. Replacement of Cl with gluconate causes a 31% decrease in Isc, while HCO₃ replacement with 20 mM HEPES (100% O₂) does not alter the Isc. Amiloride (1 μM) decreases the Isc by 43% and bumetanide (10 μM) decreases the Isc by 34%. Both 8-Br cGMP and 8-Br cAMP stimulate the Isc by 35%. Histamine, substance P and leukotriene C all increased the Isc between 16-28%. Cl replacement with gluconate blocks responses by both bumetanide and histamine. These results suggest: (i) there is a basal Cl secretion occurring across the equine trachea; (ii) Na absorption occurs across the tissue by way of an amiloride inhibitable Na channel in the apical membrane; (iii) the increase in Isc due to histamine is a result of increased Cl secretion; (iv) Cl secretion appears to be dependent on the activity of a NaCl or NaKCl cotransporter located on the basolateral membrane that is inhibited by bumetanide.

**PHARMACOKINETIC DISPOSITION OF
AN IMMEDIATE RELEASE AMINOPHYLLINE
AND A SUSTAINED RELEASE
THEOPHYLLINE FORMULATION
IN ADULT HORSES**

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The pharmacokinetic disposition of theophylline was determined in 6 horses following the administration of aminophylline IV (9.94 mg/kg as theophylline), aminophylline tablets (9.94 mg/kg as theophylline) and Theo-dur tablets (20 mg/kg as theophylline). Mean values for the elimination rate constant (λ_z), apparent volume of distribution (V_z) and clearance (CL) determined by compartmental analysis for the IV data were 0.0716 ± 0.0135 /h, 0.8011 ± 0.0618 l/kg and 0.0572 ± 0.0109 l/kg/h, respectively. Values for the mean residence time determined by non-compartmental analysis of the oral data were significantly different for the rapid release aminophylline tablets and sustained release Theo-dur tablets (13.8 ± 2.8 h and 18.2 ± 2.3 h, respectively). Aminophylline tablets rapidly achieved peak theophylline plasma concentrations of 11.51 ± 1.4 μ g/ml within 1.6 ± 0.6 h while Theo-dur tablets were slowly absorbed and achieved peak theophylline plasma concentrations of 17.20 ± 1.3 μ g/ml within 7.3 ± 1.0 h. Absolute bioavailability was 87% and 97% for aminophylline and Theo-dur, respectively. The principle of superposition was used to predict theophylline plasma concentrations to provide a dosage regimen for Theo-dur. A loading dose of 20 mg/kg followed by maintenance doses of 15 mg/kg q. 25 h is predicted to achieve trough-peak theophylline plasma concentrations between 6 and 17 μ g/ml in adult horses.

**CAPTOPRIL PROVIDES SUSTAINED
HEMODYNAMIC BENEFITS IN DOGS WITH
EXPERIMENTALLY INDUCED
MITRAL REGURGITATION**

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Mitral regurgitation (MR) often leads to congestive heart failure which is aggravated by compensatory responses mediated by the sympathetic nervous system and the renin-angiotensin-aldosterone system. These mechanisms contribute to increased total peripheral resistance (TPR) and retention of sodium and water. The purpose of this study is to evaluate the immediate and long-term hemodynamic effects of captopril, an angiotension converting enzyme inhibitor. Ten dogs were randomly divided into a treatment (TRT) and control (CON) group after surgical creation of MR. Captopril therapy (2 mg/kg orally TID) was initiated in the TRT group after measurements of baseline hemodynamic parameters (HPs) at 2 days postoperatively, whereas the CON group received no therapy during the study. The HPs were reevaluated at 6 and 22 weeks. Changes in HPs were analyzed using a two-way analysis of variance (SAS GLM). The forward ejection fraction (FEF) of the TRT group increased slightly, whereas the regurgitant stroke volume (RSV) and TPR decreased. In contrast, the FEF decreased in the CON group, whereas the RSV and TPR increased. Changes in FEF, RSV, and TPR were significantly different ($p < 0.1$) between the two groups. The change in FEF significantly correlated with the change in TPR ($r = -0.5$ $p < 0.03$) in both groups. Although the most dramatic changes were observed at 6 weeks, the difference between groups persisted throughout the study, indicating that captopril administration resulted in sustained hemodynamic improvement and may, therefore, delay the onset of congestive heart failure in dogs with MR.

**DISPOSITION KINETICS OF INTRAVENOUSLY
ADMINISTERED CAFFEINE
IN DOGS WITH
ACUTE LIVER DISEASE**

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Disposition kinetics of intravenously administered caffeine (20mg/kg) were compared between 6 control beagle dogs and 6 beagle dogs with acute liver disease. Following pre-treatment evaluation, acute hepatic necrosis was induced by the oral administration of dimethylnitrosamine (DMNA), a liver specific toxin. Severity of liver disease was monitored using laboratory tests. Plasma clearance of indocyanine green was measured just prior to caffeine kinetic studies to estimate changes in hepatic blood flow. Caffeine serum samples were collected for 36 hours. Following kinetic studies, animals were sacrificed and liver tissue was harvested for histopathology. Serum caffeine was measured by high performance liquid chromatography. Kinetic data were analyzed using computer nonlinear regression programs which utilize least-squares for estimation of best fit. Volume of distribution at steady state (V_{dss}), clearance (Cl) and mean residence time (MRT) were calculated using area under the curve and area under the moment curve. Student's t test was used for group mean comparison and Pearson's coefficient for correlation between kinetic parameters and laboratory tests ($p \leq 0.050$). ICG MRT was significantly prolonged in the acute group. Caffeine disposition parameters for the control and acute group respectively were as follow's: V_{dss} (l/kg) 0.78 ± 0.04 and 0.72 ± 0.07 ; Cls (ml/min/kg) 1.79 ± 0.60 and 2.34 ± 0.92 and MRT (min) 402.6 ± 111 and 368.5 ± 214 . There were no significant differences in V_{dss} , Cls or MRT between groups. Caffeine MRT and Cls could be correlated to several laboratory tests indicative of liver disease.

**EARLY PHARMACOLOGICAL AND
ELECTROPHYSIOLOGICAL CHANGES
FOLLOWING EXPOSURE TO
ORGANOPHOSPHATES INDUCING DELAYED
NEUROPATHY (OPIDN)**

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Some of the organophosphorus esters used today in agriculture and industry have potential to induce a delayed neuropathy in man and other susceptible animal species following a single exposure. The present study was undertaken to characterize early changes occurring prior to clinical and histological manifestations typically used to define OPIDN. Tri-o-cresyl phosphate (TOCP, 360 mg/kg po) and phenyl saligenin phosphate (PSP, 2.5 mg/kg im) were administered to 2 groups of adult white leghorn hens, 35 in each group. These were sacrificed on days 4, 7, 10, 15, 21, and 37 following exposure for pharmacological and electrophysiological evaluation. Untreated hens served as controls. Denervation hypersensitivity of biventer cervicis nerve-muscle preparations to acetylcholine (ACh) was evident as early as 4 days following TOCP or PSP treatment. The magnitude of sensitivity differences from control were 5x at 4 days, 10⁶x at 21 days, and 5x at 37 days. Strength-duration-curves (SDC) showed discontinuities and increases in threshold excitability at 4 days following treatment with either TOCP or PSP. The early changes in SDC and responses to ACh indicate partial denervation, which progressed to complete denervation at 15 and 21 days. SDC at 37 days indicated partial reinnervation. The pattern of neurite degeneration and regeneration was further apparent on histopathological examination. Clinical signs of OPIDN were not significant before day 10, these studies would therefore indicate the usefulness of pharmacological and electrophysiological assessment before such signs develop. (Supported by NIEHS 03384).

