Pharmacokinetics of pentoxifylline and its 5-hydroxyhexyl metabolite after intravenous administration of increasing doses to sheep

Orhan Corum DVM, PhD
Duygu Durna Corum DVM, PhD
Orkun Atik DVM
Ayse Er DVM, PhD
Kamil Uney DVM, PhD

Received August 7, 2018.
Accepted October 30, 2018.

From the Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, University of Kastamonu, 37200, Kastamonu, Turkey (O Corum, DD Corum); the Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, University of Afyon Kocatepe, 03200, Afyonkarahisar, Turkey (Atik); and the Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, University of Selcuk, 42031, Konya, Turkey (Er, Uney).

Address correspondence to Dr. Corum (orhancorum46@hotmail.com).

OBJECTIVE
To determine the pharmacokinetics of pentoxifylline (PTX) and its 5-hydroxyhexyl metabolite (M-1) after IV administration of increasing doses of PTX to sheep.

ANIMALS
6 healthy adult Merino sheep.

PROCEDURES
Each sheep received 10-, 20-, and 40-mg/kg doses of PTX, IV, with a 15-day washout period between doses. Blood samples were collected before and at predetermined times after administration of each dose to determine plasma PTX and M-1 concentrations by high-performance liquid chromatography. Pharmacokinetic parameters for PTX and M-1 were estimated by noncompartmental analysis.

RESULTS
No adverse effects were observed after administration of the 10- and 20-mg/kg doses. Following administration of the 40-mg/kg dose, all sheep developed tachycardia and hypersalivation and appeared agitated for approximately 4 hours. Plasma PTX concentrations considered therapeutic in other species were achieved in all sheep after administration of all 3 doses. Pharmacokinetic parameters for PTX and M-1 varied in a dose-dependent linear manner. For PTX, the mean area under the concentration-time curve (AUC), elimination half-life, and volume of distribution increased with dose and ranged from 15.67 to 94.66 h•μg/mL, 0.68 to 0.91 hours, and 0.55 to 0.66 L/kg, respectively, whereas clearance decreased with dose and ranged from 0.42 to 0.64 L/h/kg. The mean ratio of the AUC for M-1 to AUC for PTX ranged from 0.38 to 0.46.

CONCLUSIONS AND CLINICAL RELEVANCE
Results indicated that pharmacokinetic parameters for PTX and M-1 varied in a dose-dependent linear manner in healthy sheep. Further studies are warranted to determine the therapeutic threshold and optimal dosage for PTX in sheep. (*Am J Vet Res* 2019;80:702–708)

ABBREVIATIONS

AUC	Area under the plasma concentration–time curve
$AUC_{0-\infty}$	Area under the plasma concentration–time curve from time 0 extrapolated to infinity
$AUC_{extrap\%}$	Area under the plasma concentration–time curve from the last measured time extrapolated to infinity and expressed as a percentage of the total area under the plasma concentration–time curve
AUC_{M-1}:AUC_{PTX}	Ratio of the area under the plasma concentration–time curve for the 5-hydroxyhexyl metabolite of pentoxifylline to the area under the plasma concentration–time curve for pentoxifylline
Cl	Total body clearance
C_{max}	Maximum plasma drug concentration
HPLC	High-performance liquid chromatography
M-1	5-hydroxyhexyl metabolite of pentoxifylline
MRT	Mean residence time
PTX	Pentoxifylline
$t_{1/2}$	Elimination half-life
t_{max}	Time to maximum plasma drug concentration
Vd_{ss}	Apparent volume of distribution at steady state

The methylxanthine derivative PTX decreases platelet adhesion to blood vessel walls, decreases blood viscosity by increasing the flexibility and deformability of RBCs, promotes fibrinolysis by decreasing plasma fibrinogen concentration, and increases the filterability of monocytes and polymorphonuclear leukocytes. Because of those effects, PTX is classified as a hemorheological agent.[1,2] Pentoxifylline also increases intracellular cAMP concentration by inhibition of the phosphodiesterase enzymes that hydrolyze cAMP.[3] Increased concentrations of cAMP prevent platelet aggregation and cyclooxygenase activity[4] and inhibit the production of cytokines, such as tumor necrosis factor-α and interleukins-1β and -6.[5] In human medicine, PTX is used to treat various diseases, including intermittent lameness, endotoxemia, vasculitis, sepsis, diabetic ulcerations, seizure disorders, cancer, and collagen disorders.[6] In veterinary medicine, PTX is used in an extralabel manner to treat dermatomyositis, vasculitis, contact allergy,

atopy, and systemic lupus erythematosus in dogs[7] and endometritis, placentitis, laminitis, and dermal vasculitis in horses.[8]

Pentoxifylline is extensively metabolized in humans and animals. One of the major metabolites of PTX in plasma is M-I, which is formed by reduction in RBCs and the liver.[9] The pharmacological activity of M-I is similar to that of PTX, and the metabolism of M-I is rapid and reversible.[10] Both PTX and M-I bind to the outer membrane of RBCs, which is the primary site for the conversion of PTX to M-I.[11] The M-I is an investigational compound under development for treatment of type I diabetes and prevention of treatment-related toxicoses in cancer patients and bone marrow transplant recipients.[12–14] The therapeutic effects of PTX and M-I in humans and horses are reportedly dose dependent.[15,16]

Pharmacokinetic studies of PTX have been conducted in horses,[7,17] dogs,[18] broiler chickens,[19] rabbits,[20] rats,[21] and mice.[22] Although various doses (10 to 60 mg/kg) of PTX have been investigated for treatment of diseases such as endotoxemia, septic shock, bronchopulmonary injury, and preeclampsia in sheep,[23–26] pharmacokinetic data for PTX in this species are lacking. Therefore, the purpose of the study reported here was to determine the pharmacokinetics of PTX and M-I following IV administration of increasing doses (10, 20, and 40 mg/kg) of PTX to sheep.

Materials and Methods

Animals

All study procedures were reviewed and approved by the Ethics Committee of the Faculty of Veterinary Medicine, University of Selcuk, Konya, Turkey. Six healthy adult Merino sheep with a mean ± SD age of 1.6 ± 0.3 years and body weight of 57 ± 4 kg were used for the study. The sheep were not administered any drugs within the 30 days prior to study initiation. The sheep were maintained under optimal nutritional conditions and were fed high-quality alfalfa hay and a drug-free concentrate that included the following: barley flakes, corn mash, dried sunflower seed meal, thick wheat bran, and a vitamin-mineral mixture (metabolic energy, 2,750 kcal/kg; dry matter, 88%; crude protein, 12%; crude fiber, 12%; vitamin A, 7,000 U/kg; vitamin D3, 700 U/kg; vitamin E, 25 mg/kg; calcium, 0.6% to 1.6%; phosphorus, 0.4%; and sodium, 0.1% to 0.4%). Sheep were fed the described ration on a daily basis and had ad libitum access to water. A physical examination was performed on each sheep on a daily basis until 7 days after administration of the last dose of PTX.

Experimental design and sampling

The study had a crossover design. Each sheep received PTX at each of 3 doses (10, 20, and 40 mg/kg), IV, with a 15-day washout period between doses. Prior to study initiation, a randomization method was used such that each PTX dose was administered to 2 sheep during each of the 3 treatment periods. Immediately before each PTX injection, a catheter was aseptically placed in the right jugular vein of each sheep for collection of serial blood samples, and PTX powder[a] was dissolved in physiologic saline (0.9% NaCl) solution in an amount sufficient to achieve a suspension with a PTX concentration of 50 mg/mL. The designated dose of PTX was injected as a bolus into the left jugular vein over 1 minute. From each sheep, a blood sample (3 mL) was obtained via the catheter in the right jugular vein immediately before (0 minutes) and at 2, 6, 10, 15, 20, 25, 30, and 45 minutes and 1, 1.5, 2, 3, 4, 5, 6, 7, 8, 10, and 12 hours after PTX administration. Each blood sample was collected into a blood collection tube containing heparin as an anticoagulant and was centrifuged at 3,500 × *g* for 15 minutes within 1 hour after collection. The plasma was harvested from each sample, placed in a cryovial, and stored frozen at –70°C until analysis. The PTX and M-I concentrations were determined for each plasma sample within 2 months after collection.

Quantification of plasma PTX and MI concentrations

For each plasma sample, the PTX and M-I[b] concentrations were determined by use of a modified HPLC method as described.[18] The HPLC system[c] was equipped with a pump, autosampler, degasser, column oven, and UV detector set at 275 nm. Pure samples of PTX and M-I were used for quality controls. For each sample, 300 µL of methanol[d] was added to a 200-µL aliquot of plasma. The resulting mixture was vortexed for 30 seconds and centrifuged at 10,000 × *g* for 10 minutes. The clear supernatant was transferred to an injection vial, and 10 µL of the supernatant was injected onto the HPLC column. The mobile phase consisted of methanol and 0.025M sodium acetate buffer[d] (40:60 v/v) with an isocratic flow rate of 1 mL/min. Chromatographic separation was performed on a C18 column[c] (length, 250 mm; internal diameter, 4.6 mm; particle size, 5 µm), which was maintained at 40°C. Pentoxifylline and M-I eluted at approximately 7.5 and 11.5 minutes, respectively, with a total run time of 15 minutes.

Blank sheep plasma samples (ie, plasma samples obtained from sheep before PTX administration) underwent HPLC analysis to ensure that no plasma-derived peaks interfered with the retention times of PTX and M-I on the chromatogram and validate the HPLC method. Calibration curves were created for both PTX and M-I at concentrations ranging from 0.04 to 100 µg/mL, and the correlation coefficients were > 0.9997 and > 0.9995 for the PTX and M-I calibration curves, respectively. The mean ± SD recovery of PTX from plasma was 98.38 ± 3.47%, and the mean ± SD recovery of M-I from plasma was 97.21 ± 4.01%. The limit of quantification was 0.04 µg/mL for both PTX and M-I. The intraday and interday coefficients of variation were ≤ 4.70% and ≤ 5.67%, respectively,

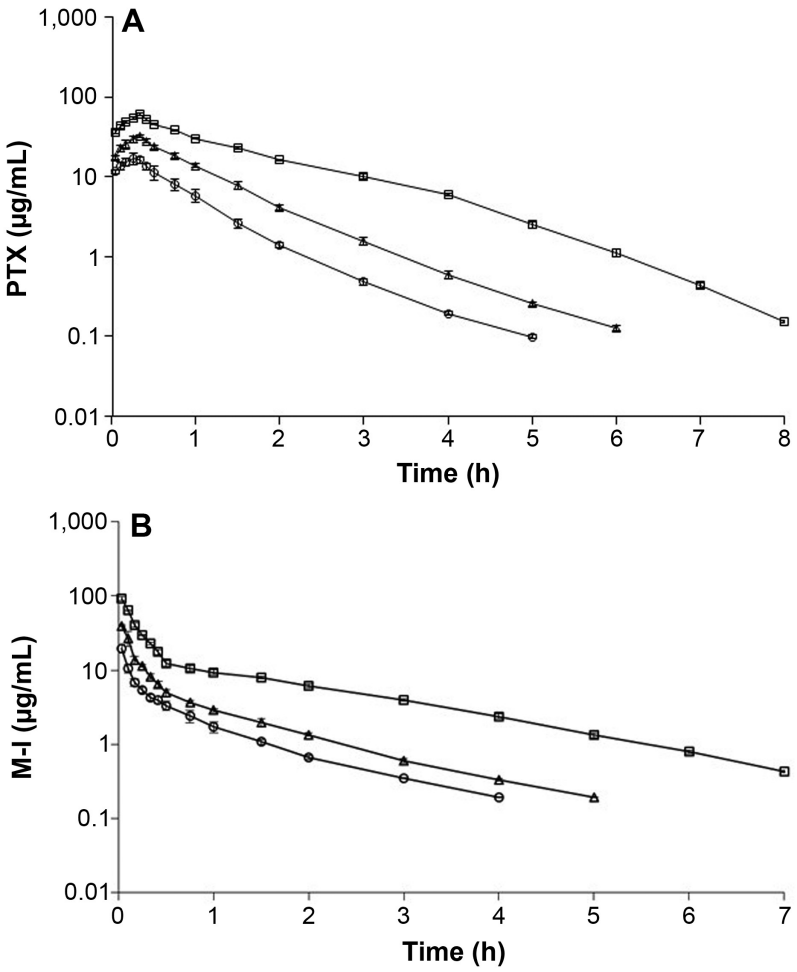

Figure 1—Mean ± SD plasma PTX (A) and M-I (B) concentrations over time for 6 healthy adult sheep following IV administration of PTX at doses of 10 mg/kg (circles), 20 mg/kg (triangles), and 40 mg/kg (squares). All sheep received each dose of PTX once in a randomized order with a 15-day washout period between doses.

for PTX and ≤ 3.82% and ≤ 4.58%, respectively, for M-I.

Pharmacokinetic analysis

A pharmacokinetic software program[f] was used to estimate pharmacokinetic parameters for PTX and M-I by noncompartmental methods. Pharmacokinetic parameters estimated for PTX included the AUC, $AUC_{extrap\%}$, $t_{1/2}$, Cl, MRT, and Vd_{ss}, and those estimated for M-I included AUC, $AUC_{extrap\%}$, and MRT. The AUC was estimated by use of the linear-log trapezoid method. The elimination rate constant was determined by linear regression analysis of the terminal portion of the log-linear plasma concentration–time curve, and the $t_{1/2}$ was calculated as ln 2/elimination rate constant. For both PTX and M-I, the C_{max} and t_{max} were determined directly from the data.

Statistical analyses

Results for $t_{1/2}$ and MRT were reported as the harmonic mean ± SD, and the results for t_{max} were reported as the median. For both PTX and M-I, Wilcoxon rank sum tests were used to compare $t_{1/2}$, MRT, and t_{max} among the 3 doses of PTX administered (10, 20, and 40 mg/kg). The mean ± SD was reported for all other pharmacokinetic parameters (ie, parameters that were normally distributed). For each normally distributed parameter, the effect of dose on that parameter was assessed by means of a 1-way ANOVA followed by the Duncan test when post hoc pairwise comparisons among the 3 doses were warranted. The C_{max} and AUC were normalized to the 10-mg/kg dose of PTX prior to ANOVA. Linear regression analyses and graphical examination were also used to evaluate the relationship between each pharmacokinetic parameter and PTX dose. All analyses were performed with statistical software,[g] and values of $P < 0.05$ were considered significant.

Results

Safety

No local or systemic adverse effects were observed in any of the sheep following IV administration of PTX at doses of 10 and 20 mg/kg. Following IV administration of the 40-mg/kg dose of PTX, all sheep developed clinically important tachycardia and hypersalivation and appeared agitated for approximately 4 hours.

Pharmacokinetic parameters

The mean ± SD plasma concentrations of PTX and M-I over time following IV administration of PTX at doses of 10, 20, and 40 mg/kg were plotted **(Figure 1)**. The pharmacokinetic parameters of PTX and M-I were summarized **(Table 1)**. For PTX, the $t_{1/2}$, $AUC_{0-\infty}$, MRT, and Cl varied significantly among the 3 doses. The mean $t_{1/2}$, $AUC_{0-\infty}$, and MRT increased, whereas the mean Cl decreased, in a linear manner as the dose increased. The dose-normalized C_{max} for PTX when the drug was administered at the 10-mg/kg dose was significantly lower than that when the drug was administered at the 40-mg/kg dose. The dose-normalized C_{max} for PTX when the drug was administered at the 20-mg/kg dose did not differ significantly from the dose-normalized C_{max} for the 10-mg/kg dose or 40-mg/kg dose. The mean Vd_{ss} for PTX ranged from 0.55 to 0.66 L/kg and was significantly greater for the 40-mg/kg dose than for the 10-mg/kg and 20-mg/kg doses.

For M-I, the $t_{1/2}$ varied significantly among the 3 doses (Table 1). The mean $t_{1/2}$ for M-I increased in a linear manner as the dose of PTX administered

Table 1—Estimated pharmacokinetic parameters for PTX and M-I in 6 healthy adult sheep following IV administration of PTX at doses of 10, 20, and 40 mg/kg.

		Dose (mg/kg)		
Analyte	**Parameter**	**10**	**20**	**40**
PTX	$t_{1/2}$ (h)*	0.68 ± 0.04[a]	0.76 ± 0.02[b]	0.91 ± 0.01[c]
	$AUC_{0-\infty}$ (h•µg/mL)	15.67 ± 1.43[a]	34.89 ± 1.60[b]	94.66 ± 1.53[c]
	$AUC_{extrap\%}$ (%)	0.64 ± 0.12	0.40 ± 0.05	0.21 ± 0.01
	MRT (h)*	0.85 ± 0.04[a]	1.01 ± 0.02[b]	1.57 ± 0.02[c]
	Cl (L/h/kg)	0.64 ± 0.06[c]	0.57 ± 0.03[b]	0.42 ± 0.01[a]
	Vd_{ss} (L/kg)	0.55 ± 0.07[a]	0.58 ± 0.03[a]	0.66 ± 0.01[b]
	C_{max} (µg/mL)	17.41 ± 2.17[a]	32.95 ± 1.13[a,b]	60.95 ± 1.33[b]
	t_{max} (h)†	0.29	0.33	0.33
M-I	$t_{1/2}$ (h)*	1.11 ± 0.06[a]	1.22 ± 0.07[b]	1.32 ± 0.03[c]
	$AUC_{0-\infty}$ (h•µg/mL)	6.82 ± 0.56[a]	13.18 ± 0.69[a]	43.58 ± 0.82[b]
	$AUC_{extrap\%}$ (%)	4.58 ± 0.56	2.59 ± 0.27	1.87 ± 0.14
	MRT (h)*	1.02 ± 0.05[a]	0.96 ± 0.05[a]	1.52 ± 0.03[b]
	C_{max} (µg/mL)	19.48 ± 2.52[a]	39.76 ± 1.22[a]	92.02 ± 5.42[b]
	t_{max} (h)†	0.33	0.33	0.33
	AUC_{M-I}:AUC_{PTX}	0.44 ± 0.03	0.38 ± 0.02	0.46 ± 0.01

Values represent mean ± SD unless otherwise indicated. All sheep received each dose of PTX once in a randomized order with a 15-day washout period between doses.

*Values represent harmonic mean ± SD. †Values represent the median.

[a–c]Within a row, values with different superscript letters differ significantly ($P < 0.05$).

increased. The median t_{max} for M-I was 0.33 hours for all 3 doses. For both PTX and M-I, the $AUC_{0-\infty}$ increased linearly with dose, and the coefficient of determination (R^2) for the linear relationship between $AUC_{0-\infty}$ and dose was 0.9887 for PTX and 0.9697 for M-I **(Figure 2)**.

Discussion

Results of the present study indicated that IV administration of PTX at doses of 10 and 20 mg/kg did not elicit any local or systemic adverse effects in healthy adult sheep, but IV administration of PTX at a dose of 40 mg/kg resulted in clinically relevant tachycardia, hypersalivation, and agitation for approximately 4 hours in all sheep. The adverse effects associated with administration of the 40-mg/kg dose may have been associated with rapid IV injection (1 minute) of PTX as a bolus that led to rapid oversaturation of drug receptor sites. Thus, the adverse effects associated with IV administration of the 40-mg/kg dose could likely be minimized or alleviated by administration of PTX as a slow IV bolus (2 to 5 minutes) or as a continuous infusion. However, in horses, injection of 8.5 mg of PTX/kg as a slow IV bolus over 2 to 5 minutes results in muscle fasciculation, sweating at the shoulders and flanks, and an increase in heart rate.[8] In chickens, PTX (100 mg/kg, IV or PO) administration causes transient somnolence, closing of the eyes, and deep respiration.[19] Conversely, in dogs, administration of PTX at doses ranging from 10 to 30 mg/kg, IV or PO, is associated with no adverse effects.[18,27]

In the present study, most of the pharmacokinetic parameters increased or decreased in a linear manner as the dose of PTX administered increased. The mean $t_{1/2}$ of PTX following IV administration to sheep ranged from 0.68 hours (10-mg/kg dose) to 0.91 hours (40-mg/kg dose), which was similar to the mean $t_{1/2}$ of PTX in humans (0.84 hours),[28] longer than the mean $t_{1/2}$ of PTX in horses (0.38 hours)[8] and dogs (0.28 hours),[18] and shorter than the mean $t_{1/2}$ of PTX in chickens (1.05 hours).[19] The mean Vd_{ss} of PTX for the sheep of the present study ranged from 0.55 L/kg (10-mg/kg dose) to 0.66 L/kg (40-mg/kg dose), which was lower than the mean Vd_{ss} reported for horses (1.15 L/kg)[8] and dogs (1.02 L/kg).[18] Likewise, the mean Cl of PTX in sheep (range, 0.42 L/h/kg [40-mg/kg dose] to 0.64 L/h/kg [10-mg/kg dose]) was lower than the mean Cl of PTX in horses (2.38 L/h/kg),[8] dogs (2.22 L/h/kg),[18] and chickens (1.90 L/h/kg).[19] The mean $t_{1/2}$ of M-I for the sheep of the present study (range, 1.11 hours [10-mg/kg dose] to 1.32 hours [40-mg/kg]) was longer than the mean $t_{1/2}$ of M-I for chickens (0.78 hours)[19] and rats (0.66 hours).[21] Differences in pharmacokinetic parameters for PTX and M-I among species may be associated with the health status and age of individuals, intervals between PTX administration and blood sample collection, analytic method used to determine PTX or M-I concentration, and interspecies variation.[29]

In humans, PTX is metabolized into 7 phase-I metabolites, expressed as M-I to M-VII.[30] Pentoxifylline is converted to M-I via reduction in RBCs and the liver and to other metabolites via oxidation in the liver.[9] Because PTX and M-I are almost completely converted to other metabolites, only 1% of the administered dose of PTX is excreted as PTX or M-I in the urine.[31] Clearance of PTX is dependent on hepatic blood flow and metabolism.[30,32,33]

For both PTX and M-I, the mean $t_{1/2}$ and Vd_{ss} increased, whereas the mean Cl decreased, as the dose of PTX administered to the sheep of the pres-

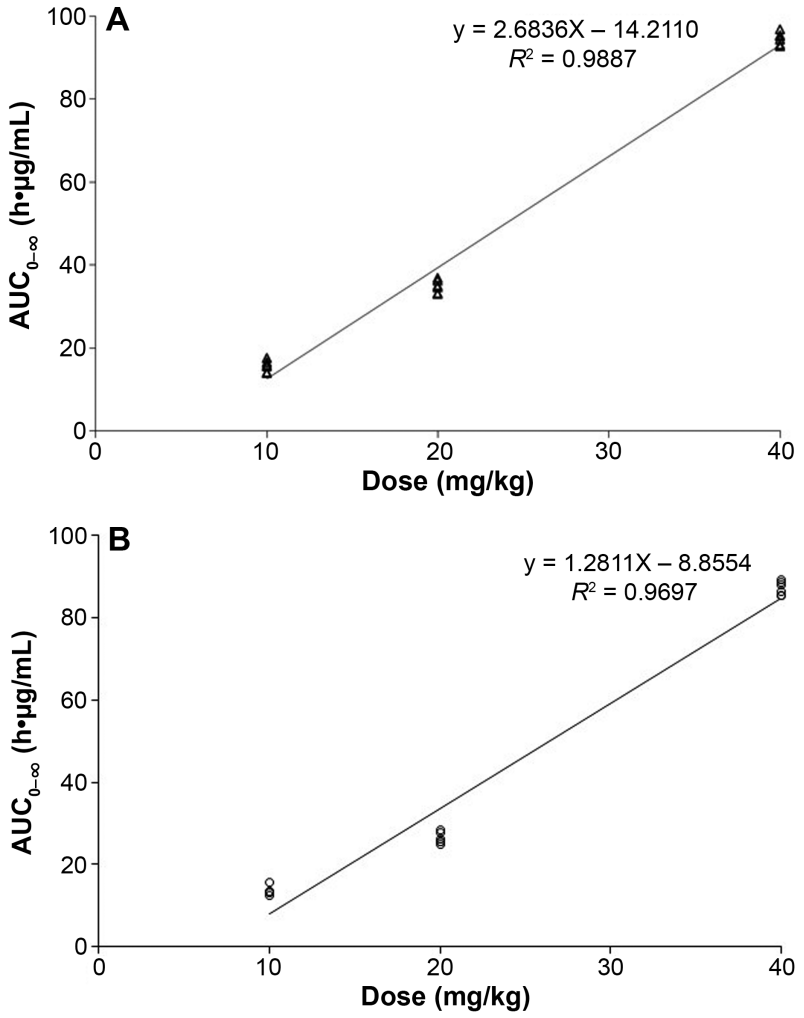

Figure 2—Scatterplots of the $AUC_{0-\infty}$ versus dose for PTX (A) and M-I (B) for the sheep of Figure 1. Each symbol represents the estimated $AUC_{0-\infty}$ for 1 sheep. Notice that the $AUC_{0-\infty}$ did not vary significantly among sheep at any of the 3 doses. Also, for both PTX and M-I, there was a strong positive linear relationship (black lines) between $AUC_{0-\infty}$ and dose, as evidenced by the provided linear regression equations and coefficients of determination (R^2). ***See*** Figure 1 for remainder of key.

ent study increased from 10 to 40 mg/kg. In humans, approximately 70% of the administered dose of PTX becomes bound to plasma proteins.[1] In the present study, the positive association between Vd_{ss} and PTX dose might have been the result of saturation of binding to the plasma proteins as the dose increased such that a greater amount of the drug remained unbound to plasma proteins and was free to diffuse into the tissues. Pentoxifylline typically increases hepatic blood flow. Therefore, it was expected that the Cl of PTX would increase as the dose increased, but the opposite was observed for the sheep of the present study. It is possible that the higher doses of PTX exceeded the capacity of the liver to metabolize the drug, which resulted in a dose-dependent decrease in Cl. In the present study, the mean $t_{1/2}$ of M-I was approximately 1.6 times the mean $t_{1/2}$ of PTX, which might have been the result of continued conversion of PTX to M-I in RBCs. Similar to the results of the present study, administration of increasing doses of PTX to rats is associated with an increase in the mean $t_{1/2}$ for both PTX and M-I and decrease in the mean Cl of PTX.[22]

In the present study, the dose-normalized mean $AUC_{0-\infty}$ for PTX differed significantly among the 3 doses, whereas the dose-normalized mean $AUC_{0-\infty}$ for M-I was significantly greater for the 40-mg/kg dose, compared with the dose-normalized mean $AUC_{0-\infty}$s for the 10- and 20-mg/kg doses, which did not differ significantly. Nevertheless, there was a linear relationship between dose and $AUC_{0-\infty}$ for both PTX and M-I. In humans, the relationship between PTX dose and $AUC_{0-\infty}$ is linear in adults[28] but is nonlinear in children.[34] In humans, administration of PTX (300 to 600 mg, IV) results in a significant decrease in the ratio of the AUC for the M-IV metabolite to the AUC for PTX ($AUC_{M\text{-}IV}$:AUC_{PTX}, from 0.35 to 0.24) and ratio of the AUC for the M-V metabolite to the AUC for PTX ($AUC_{M\text{-}V}$:AUC_{PTX}, from 0.26 to 0.21); however, the $AUC_{M\text{-}I}$:AUC_{PTX} decreases only minimally and nonsignificantly (from 3.02 to 2.81).[11] The conversion of PTX to the M-IV and M-V metabolites occurs only in the liver, whereas the conversion of PTX to M-I occurs primarily in RBCs as well as the liver.[9] The significant decrease in the ratios for $AUC_{M\text{-}IV}$:AUC_{PTX} and $AUC_{M\text{-}V}$:AUC_{PTX} with increasing doses of PTX might reflect saturation of the hepatic capacity to convert PTX to the M-IV and M-V metabolites and an unparalleled increase in the AUC of PTX relative to that for the M-IV and M-V metabolites. The nonsignificant decrease in the $AUC_{M\text{-}I}$:AUC_{PTX} with increasing doses of PTX was likely a reflection of the interconversion of M-I to PTX in RBCs despite saturation of the hepatic capability to metabolize PTX to M-I. In the present study, the mean $AUC_{0-\infty}$ for M-I was significantly greater for the 40-mg/kg dose relative to that for both the 10- and 20-mg/kg doses. Additionally, the lowest detectable plasma concentration of PTX was at 5, 6, and 8 hours after drug administration for the 10-, 20-, and 40-mg/kg doses, respectively, whereas the lowest detectable plasma concentration of M-I was at 4, 5, and 7 hours after PTX administration for the 10-, 20-, and 40-mg/kg doses, respectively. It is possible that the increase in mean $AUC_{0-\infty}$ for both PTX and M-I as the PTX dose increased was caused by a dose-dependent decrease in Cl and changes in the plasma

concentration–time profile relative to sampling time owing to an increase in $t_{1/2}$.

For the sheep of the present study, the mean $AUC_{M\text{-}I}$:AUC_{PTX} did not differ significantly among the 3 doses of PTX administered. This finding was contrary to results observed in children; the $AUC_{M\text{-}I}$:AUC_{PTX} decreases considerably as PTX dose increases owing to enzymatic saturation.[34] The mean $AUC_{M\text{-}I}$:AUC_{PTX} for the sheep of the present study (0.38 to 0.46) was lower than that reported for horses (1.13 to 2.4),[8,17] dogs (0.63),[27] and chickens (1.52).[19] As previously stated, the conversion of PTX to M-I occurs in both RBCs and the liver. The binding activity of PTX to the RBC membrane is 45%, and PTX is converted to M-I via aldo-keto reductase.[1,h] That conversion, which is reversible, was likely responsible for the increase in plasma PTX concentration, which for the sheep of the present study peaked at approximately 20 minutes after injection of PTX as an IV bolus. The major active metabolites of PTX in plasma are M-I and M-V in humans,[11] chickens,[19] and dogs,[27] and in those species, the plasma concentrations of M-I and M-V are generally greater than the corresponding plasma concentration of PTX. However, in another study involving dogs,[18] the plasma M-V concentration was not detectable, and the plasma M-I concentration was less than the corresponding plasma PTX concentration. Aldo-keto reductase activity varies among species[35]; therefore, the plasma M-I concentration may vary among species following administration of the same dose of PTX. The low $AUC_{M\text{-}I}$:AUC_{PTX} for the sheep of the present study might have been the result of low aldo-keto reductase activity or the conversion of PTX to metabolites other than M-I, as has been observed in dogs[27] and rats.[21]

For the sheep of the present study, the mean C_{max} for M-I was 19.48, 39.76, and 92.02 µg/mL following IV administration of PTX at doses of 10, 20, and 40 mg/kg, respectively. When standardized for dose, the mean C_{max} for M-I observed for the sheep of the present study was greater than the mean C_{max} for M-I following IV administration of PTX at a dose of 8.5 mg/kg to horses (5.6 µg/mL)[8] and 100 mg/kg to chickens (43.93 µg/mL).[19]

The pharmacodynamics of PTX vary in a dose-dependent manner. The therapeutic concentration of PTX ranges from 0.5 to 2 µg/mL in humans.[16] In an in vitro model, release of tumor necrosis factor-α in response to endotoxin was inhibited in equine blood samples spiked with PTX at concentrations ranging from 1 to 10 µg/mL.[15] In the present study, the mean C_{max} for PTX was 17.41, 32.95, and 60.95 µg/mL following IV administration of the drug at doses of 10, 20, and 40 mg/kg, respectively. Moreover, the plasma PTX concentration remained > 10 µg/mL for 0.5, 1, and 3 hours and > 0.5 µg/mL for 3, 4, and 6 hours following administration of the 10-, 20-, and 40-mg/kg doses, respectively. Thus, the plasma PTX concentration achieved for the sheep of this study exceeded the therapeutic threshold reported for humans[16] and presumed in vivo therapeutic threshold for horses[15] at all 3 doses administered. Also, the duration that the plasma PTX concentration exceeded the therapeutic threshold varied in a dose-dependent manner. Results of experimental studies indicate that PTX has beneficial effects in sheep with endotoxemia,[23] septic shock,[25] bronchopulmonary injury,[24] and preeclampsia.[26] Thus, we believe that administration of repeated doses of PTX might be useful for the treatment of sheep with those diseases in clinical settings.

The pharmacokinetic parameters of PTX and M-I varied in a dose-dependent linear manner following IV administration of PTX at doses of 10, 20, and 40 mg/kg to healthy sheep. No adverse effects were associated with administration of PTX at doses of 10 and 20 mg/kg, but administration of the 40-mg/kg dose was associated with clinically important tachycardia and hypersalivation, and all sheep appeared agitated for approximately 4 hours after PTX injection. Those adverse effects might be minimized or avoided by administration of PTX as a slow IV bolus over several minutes or as a continuous infusion. However, plasma PTX concentrations considered therapeutic in other species were achieved in all sheep following administration of the 10- and 20-mg/kg doses. Therefore, we believe that sheep can be safely administered PTX at doses of 10 and 20 mg/kg. The therapeutic threshold of PTX varies among species; thus, further studies are warranted to determine the therapeutic threshold and optimal dosage for PTX in sheep with various diseases.

Acknowledgments

The authors declare that there were no conflicts of interest.

Presented in poster form at the V. International KOP Regional Development Symposium, Konya, Turkey, October 2017.

Footnotes

a. PTX (assay purity, ≥ 99%), Sigma-Aldrich, St Louis, Mo.
b. M-I (assay purity, ≥ 99%), Sigma-Aldrich, St Louis, Mo.
c. Shimadzu, Tokyo, Japan.
d. Merck, Darmstadt, Germany.
e. Gemini C18 analytic column, Phenomenex, Torrance, Calif.
f. Phoenix WinNonlin, version 6.1.0.173, Certara, Princeton, NJ.
g. SPSS, version 22.0, IBM Corp, Armonk, NY.
h. Magnusson M. *Pharmacokinetics and pharmacodynamics of pentoxifylline and metabolites in humans*. PhD thesis, Faculty of Medicine, Lund University, Lund, Sweden, 2009.

References

1. Harris E, Schulzke SM, Patole SK. Pentoxifylline in preterm neonates: a systematic review. *Paediatr Drugs* 2010;12:301–311.
2. Schröer RH. Antithrombotic potential of pentoxifylline, a hemorrheologically active drug. *Angiology* 1985;36:387–398.
3. Bessler H, Gilgal R, Djaldetti M, et al. Effect of pentoxifylline on the phagocytic activity, cAMP levels, and superoxide anion production by monocytes and polymorphonuclear cells. *J Leukoc Biol* 1986;40:747–754.
4. Sha MC, Callahan CM. The efficacy of pentoxifylline in the treatment of vascular dementia: a systematic review. *Alzheimer Dis Assoc Disord* 2003;17:46–54.

5. Ward A, Clissold SP. Pentoxifylline. A review of its pharmacodynamic and pharmacokinetic properties, and its therapeutic efficacy. *Drugs* 1987;34:50–97.
6. Samlaska CP, Winfield EA. Pentoxifylline. *J Am Acad Dermatol* 1994;30:603–621.
7. Sykes JE, Papich MP. Antiviral and immunomodulatory drugs. In: Sykes JE, ed. *Canine and feline infectious diseases*. St Louis: Elsevier Saunders, 2014;54–65.
8. Liska DA, Akucewich LH, Marsella R, et al. Pharmacokinetics of pentoxifylline and its 5-hydroxyhexyl metabolite after oral and intravenous administration of pentoxifylline to healthy adult horses. *Am J Vet Res* 2006;67:1621–1627.
9. Aviado DM, Porter JM. Pentoxifylline: a new drug for the treatment of intermittent claudication. Mechanism of action, pharmacokinetics, clinical efficacy and adverse effects. *Pharmacotherapy* 1984;4:297–307.
10. Lee SH, Slattery JT. Cytochrome P450 isozymes involved in lisofylline metabolism to pentoxifylline in human liver microsomes. *Drug Metab Dispos* 1997;25:1354–1358.
11. Nicklasson M, Björkman S, Roth B, et al. Stereoselective metabolism of pentoxifylline in vitro and in vivo in humans. *Chirality* 2002;14:643–652.
12. List AF, Maziarz R, Stiff P, et al. A randomized placebo-controlled trial of lisofylline in HLA-identical, sibling-donor, allogeneic bone marrow transplant recipients. The Lisofylline Marrow Transplant Study Group. *Bone Marrow Transplant* 2000;25:283–291.
13. Margolin K, Atkins M, Sparano J, et al. Prospective randomized trial of lisofylline for the prevention of toxicities of high-dose interleukin 2 therapy in advanced renal cancer and malignant melanoma. *Clin Cancer Res* 1997;3:565–572.
14. Yang Z, Chen M, Nadler JL. Lisofylline: a potential lead for the treatment of diabetes. *Biochem Pharmacol* 2005;69:1–5.
15. Barton MH, Moore JN. Pentoxifylline inhibits mediator synthesis in an equine in vitro whole blood model of endotoxemia. *Circ Shock* 1994;44:216–220.
16. Regenthal R, Krueger M, Koeppel C, et al. Drug levels: therapeutic and toxic serum/plasma concentrations of common drugs. *J Clin Monit Comput* 1999;15:529–544.
17. Crisman MV, Wilcke JR, Correll LS, et al. Pharmacokinetic disposition of intravenous and oral pentoxifylline in horses. *J Vet Pharmacol Ther* 1993;16:23–31.
18. Marsella R, Nicklin CF, Munson JW, et al. Pharmacokinetics of pentoxifylline in dogs after oral and intravenous administration. *Am J Vet Res* 2000;61:631–637.
19. De Boever S, Baert K, De Backer P, et al. Pharmacokinetics and oral bioavailability of pentoxyfylline in broiler chickens. *J Vet Pharmacol Ther* 2005;28:575–580.
20. Adcock KG, Kyle PB, Deaton JS, et al. Pharmacokinetics of intranasal and intratracheal pentoxifylline in rabbits. *Pharmacotherapy* 2007;27:200–206.
21. Italiya KS, Sharma S, Kothari I, et al. Simultaneous estimation of lisofylline and pentoxifylline in rat plasma by high performance liquid chromatography-photodiode array detector and its application to pharmacokinetics in rat. *J Chromatogr B Analyt Technol Biomed Life Sci* 2017;1061–1062:49–56.
22. Honess DJ, Dennis IF, Bleehen NM. Pentoxifylline: its pharmacokinetics and ability to improve tumour perfusion and radiosensitivity in mice. *Radiother Oncol* 1993;28:208–218.
23. Chalmeh A, Rahmani Shahraki A, Heidari SM, et al. The comparative efficacy of tyloxapol versus pentoxifylline against induced acute phase response in an ovine experimental endotoxemia model. *Inflammopharmacology* 2016;24:59–64.
24. Ogura H, Cioffi WG, Okerberg CV, et al. The effects of pentoxifylline on pulmonary function following smoke inhalation. *J Surg Res* 1994;56:242–250.
25. Sigurdsson GH, Youssef H. Effects of pentoxifylline on hemodynamics, gas exchange and multiple organ platelet sequestration in experimental endotoxic shock. *Acta Anaesthesiol Scand* 1993;37:396–403.
26. Tálosi G, Németh I, Pintér S. Inhibitory effects of methylxanthines on the pre-eclamptic-like symptoms in ewes. *Eur J Obstet Gynecol Reprod Biol* 2001;99:25–32.
27. Rees CA, Boothe DM, Boeckh A, et al. Dosing regimen and hematologic effects of pentoxifylline and its active metabolites in normal dogs. *Vet Ther* 2003;4:188–196.
28. Smith RV, Waller ES, Doluisio JT, et al. Pharmacokinetics of orally administered pentoxifylline in humans. *J Pharm Sci* 1986;75:47–52.
29. Haddad NS, Pedersoli WM, Ravis WR, et al. Combined pharmacokinetics of gentamicin in pony mares after a single intravenous and intramuscular administration. *Am J Vet Res* 1985;46:2004–2007.
30. Hinze HJ. Pharmacokinetics of 3,7-dimethyl-1-(5-oxohexyl)-xanthine (BL 191) in man. *Arzneimittelforschung* 1972;22:1492–1495.
31. Beermann B, Ings R, Månsby J, et al. Kinetics of intravenous and oral pentoxifylline in healthy subjects. *Clin Pharmacol Ther* 1985;37:25–28.
32. Rames A, Poirier JM, LeCoz F, et al. Pharmacokinetics of intravenous and oral pentoxifylline in healthy volunteers and in cirrhotic patients. *Clin Pharmacol Ther* 1990;47:354–359.
33. Suren A, Bauer FE, Rosenkranz B, et al. Effect of pentoxifylline on liver plasma flow in normal man. *Eur J Clin Pharmacol* 1991;41:233–237.
34. Best BM, Burns JC, DeVincenzo J, et al. Pharmacokinetic and tolerability assessment of a pediatric oral formulation of pentoxifylline in Kawasaki disease. *Curr Ther Res Clin Exp* 2003;64:96–115.
35. Szotáková B, Baliharová V, Lamka J, et al. Comparison of in vitro activities of biotransformation enzymes in pig, cattle, goat and sheep. *Res Vet Sci* 2004;76:43–51.