Dosing Regimen and Hematologic Effects of Pentoxifylline and Its Active Metabolites in Normal Dogs*

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ABSTRACT

The disposition of pentoxifylline and two of its active metabolites (metabolite 1 [M1] and metabolite 5 [M5]) were studied following IV (8 mg/kg) and PO (30 mg/kg) administration to eight normal dogs using a randomized crossover design. Blood samples were collected at fixed time intervals after drug administration for determination of drug concentrations, platelet aggregation, and plasma fibrinogen. Complete blood counts, serum chemistry profiles, fibrinogen, and urinalysis were monitored at the beginning and end of each phase of the study (PO versus IV administration). Pentoxifylline was readily metabolized and bioavailable (50% ± 26%). Both M1 and M5 were present throughout the study, with M5 predominating. Human drug therapeutic concentrations (1,000 ng/ml) were present for 170 ± 24 minutes following IV administration and 510 ± 85 minutes after PO dosing. These findings suggest that a 12-hour dosing regimen is appropriate. None of the dogs experienced any adverse effects after pentoxifylline administration. The lack of hematologic effects suggests that the immunologic effects of pentoxifylline may be of more importance in dogs.

INTRODUCTION

Pentoxifylline is a methylxanthine drug with both hematologic and immunologic properties. Unlike most methylxanthine drugs, pentoxifylline is known for its cardiac-sparing properties (less potent chronotropic and inotropic effects on the heart than theophylline) and infrequent side effects. The drug is available as a sustained-release PO preparation (Trenal; Hoechst-Roussel Pharmaceuticals) and has been used to treat a variety of human and veterinary medical conditions. In humans, indications for pentoxifylline include intermittent claudication, vasculitis, sarcoidosis, seizure disorders, hearing
disorders, sepsis, and impaired wound healing.\textsuperscript{2} In veterinary medicine, pentoxifylline has been used to treat dermatomyositis, vasculitis, rabies-induced alopecia, German shepherd pyoderma, idiopathic cutaneous and renal glomerular vasculopathy in Greyhounds, allergic contact dermatitis, atopy, and erythema multiforme.\textsuperscript{3–5}

Clinicopathological abnormalities associated with pentoxifylline therapy in humans are limited. According to the manufacturer’s label, the most likely adverse effects include leukopenia, thrombocytopenia, and elevated liver enzymes, although the incidence of these is reportedly low.\textsuperscript{6} Additionally, pentoxifylline has been found to affect fibrinogen and platelet aggregation tests in humans, and therefore, is thought to affect clotting.\textsuperscript{1,6} The impact of pentoxifylline on hemostasis has caused concern regarding its use in veterinary medicine. Although no reports of hemostatic defects have been reported for the use of pentoxifylline in dogs, no study has actively sought evidence of potentially drug-induced hemostatic abnormalities.

Other side effects reported for pentoxifylline in humans occur in less than 3\% of patients treated with the drug and include angina or chest pain, belching/flatus/bloating, dyspepsia, nausea, vomiting, dizziness, headache, and tremor.\textsuperscript{6} Of these, only vomiting and diarrhea have been reported in dogs.\textsuperscript{7} As in humans, these side effects can be minimized by administering the drug with food.\textsuperscript{6,7}

The disposition of pentoxifylline is complicated. In humans, it is metabolized by the liver into at least seven metabolites, several of which may be active.\textsuperscript{1} Two of the metabolites (M1 and M5) (Figure 1) appear to be the most prominent and biologically active in humans and are responsible for the hematologic effects observed with this drug.\textsuperscript{2,9}

Whether the presence of these metabolites influences potency is not known. However, in humans, peak M1 concentrations are five times higher and M5 is eight times higher than peak pentoxifylline concentrations following PO administration of pentoxifylline.\textsuperscript{1,2,10,11}

A recent study in dogs reported M1, M3, and M7 (but not M5) to be present in blood after pentoxifylline administration.\textsuperscript{11} However, only M1 and pentoxifylline were directly quantitated. Based on that report, the currently recommended PO dosage of pentoxifylline is 15 mg/kg every 8 hours.\textsuperscript{12} Yet, clinical responses to pentoxifylline in dogs appear to require dosages as high as 25 to 35 mg/kg every 8 to 12 hours.\textsuperscript{7} The intent of the present study was to more accurately characterize the disposition of pentoxifylline and metabolites M1 and M5 in normal dogs following administration of pentoxifylline. Additionally, this study was designed to evaluate the hematologic effects of pentoxifylline and its active metabolites using a dosing regimen that is clinically effective.

\section*{Materials and Methods}

\textbf{Dogs and Drug Administration}

Eight healthy female hound-type dogs 2 to 5 years of age and weighing between 22 to 27 kg

![Figure 1. Chemical structure of pentoxifylline, metabolite 1 (M1) and metabolite 5 (M5), and the internal standard (theophylline).](image-url)
were obtained from the Texas A&M University Laboratory Animal Resource and Research Facility. All experimental protocols were approved by the University Laboratory Animal Care Committee, which assured compliance with federal regulations for the care and use of laboratory animals. Each dog received a single dose of pentoxifylline either IV (8 mg/kg) or PO (30 mg/kg) using a randomized crossover design with a 1-week wash out period between treatments. The IV dosage was selected based on a dose similar to that used in humans. The PO sustained-release dosage was based on an anticipated bioavailability of 50% or less and the clinical observation that 15 mg/kg is insufficient for treatment of dermatologic conditions responsive to pentoxifylline. Pentoxifylline was prepared for IV administration by dissolving a pure powder in sterile water to a concentration of 100 mg/ml. The solution was sterilized by filtration through a 0.2-µm filter. The morning of each phase of the study, a 16-gauge indwelling jugular vein catheter was aseptically placed in the external jugular vein of each dog for blood collection. Food was withheld for 12 hours before the beginning of the study. Fifteen minutes prior to each drug administration, one-quarter of a can of dog food (Prescription Diet i/d, Hill’s) was fed to each dog to minimize gastrointestinal side effects.

Blood Sample Collection

Blood samples were collected using EDTA as an anticoagulant before (Time 0) and at 15, 30, 45 minutes and 1, 1.5, 2, 3, 4, 6, 8, 12, 24, and 36 hours after pentoxifylline administration. Plasma was harvested from each sample within 1 hour after collection and frozen at −70°C until assayed. Additional blood samples were collected in tubes containing sodium citrate at 0 and 4 hours after drug administration to study whole-blood platelet aggregation and ATP release. Complete blood counts (CBCs), serum chemistries, fibrinogen, and urinalysis parameters were evaluated at the beginning and the end of each treatment period.

Determination of Plasma Pentoxifylline and Metabolites

Plasma pentoxifylline and two of its metabolites (M1 and M5) were detected and quantitated using high-performance liquid chromatography (HPLC) according to previously described methods. Briefly, 0.5 ml of plasma was combined with 4 ml of dichloromethane and 0.02 ml of theophylline as the internal standard in a Teflon (DuPont)-lined screw-cap glass test tube. The mixture was vigorously vortexed and then centrifuged at 900 × g for 15 minutes. The organic layer was evaporated to dryness using a nitrogen evaporator at 40°C and then reconstituted in 200 µl of mobile phase. The injection volume was 30 µl. Analytes were separated at ambient temperature using a C18 reverse-phase column and detected using ultraviolet spectrophotometry at 274 nm. The mobile phase consisted of a mixture of 77.9% water, 22% acetonitrile, and 0.1% acetic acid. The flow rate for the mobile phase was 0.75 ml/min. The standards for each compound (pentoxifylline, M1, and M5) were supplied by the manufacturer in a purified form. Each compound was quantitated by comparing the ratio of analyte:theophylline peak heights generated from unknown samples with the ratio measured in samples containing known concentrations of each analyte. The lower limits of quantification (LOQ) were 30 ng/ml for pentoxifylline, 10 ng/ml for M1, and 100 ng/ml for M5. Precision and accuracy, respectively, were 100.5% and 85% for pentoxifylline, 99.0% and 91% for M1, and 102.0 and 92% for M5.

Platelet Aggregation

Collagen-mediated platelet aggregation and
adenosine triphosphate (ATP) release were studied in a whole-blood aggregometer (Chrono-Log) using the 0- and 4-hour blood samples collected in tubes containing sodium citrate. Collagen (2 µg) was used as an agonist in diluted whole blood (500 µl citrated blood diluted with 475 µl of HEPES-Tyrode’s buffer and 25 µl of Chrono-lume). Collagen and reagents (Chrono-Lume, Chrono-Log) were prepared according to manufacturer specifications. Collagen was selected for platelet aggregation because it is a potent agonist and closely mimics the physiologic state in dogs.8,13

Data Analysis
For each treatment, log plasma pentoxifylline concentration verses time data were subjected to computer-assisted linear regression to determine pharmacokinetic parameters. Noncompartmental analysis was implemented using either an intravascular single bolus dose or extravascular dose model. Parameters were estimated using a linear/log trapezoidal rule with λ being estimated to infinity.13,14 Peak plasma concentration was extrapolated from the first time point for the IV data (C₀), whereas Cₘₐₓ represented the actual highest measurement at the actual time Tₘₐₓ for the PO data. Bioavailability (F) was calculated according to the equation:

\[ F = \frac{AUC_{PO} \times \text{Dose}_{IV}}{AUC_{IV} \times \text{Dose}_{PO}} \]

where AUC is the area under the curve. Clearance (Cl) was determined using the equation:

\[ Cl = \frac{\text{Dose}_{IV}}{\text{AUC}} \]

and the volume of apparent distribution (Vdₜₜ) was determined using the equation:

\[ Vd_{ss} = \frac{\text{Dose}_{IV}}{\text{AUMC}/(\text{AUC})^2} \]

where AUMC is the area under the moment curve.

Means, harmonic means (for elimination half-life) and standard deviations (pseudo-standard deviation for elimination half-life) were determined utilizing a commercial statistical software package (Win Nonlin 3.1, Pharsight). Selected pharmacokinetic parameters were compared between treatment routes (PO versus IV), and selected pharmacodynamic (hemostasis) parameters were compared before and after treatment using the paired Student’s t-test. A value was considered statistically significant when \( P < .05 \). Selected parameters were compared between metabolites and parent compound.

RESULTS
Pentoxifylline was well tolerated in all dogs. Hematologic, serum chemistry, and urinalysis parameters (including CBC, fibrinogen, and platelet aggregation) did not statistically differ in any dog following pentoxifylline administration, as compared with baseline values. Following IV administration of pentoxifylline, fibrinogen concentrations were 135 ± 15 mg/dl at Time 0 and 134 ± 13 mg/dl at 4 hours. Fibrinogen concentrations were 140 ± 16 mg/dl at Time 0 and 133 ± 20 mg/dl 4 hours after PO administration. Following IV administration of pentoxifylline, platelet aggregation was 7.8 ± 1.8 ohms/min at Time 0 and 7.6 ± 1.7 ohms/min 4 hours after drug administration. Following PO administration, platelet aggregation was 8.1 ± 1.7 ohms/min at Time 0 and 8.3 ± 1.7 ohms/min 4 hours after drug administration.

Samples were not available for one dog (Dog 8) following IV administration of pentoxifylline. However, PO data was available for this dog and is included in this report. Concentrations of pentoxifylline achieved were above those considered therapeutic in humans (1,000 ng/ml) and were maintained for 170 ± 24 minutes (IV) and 510 ± 24 minutes (oral). The mean concentrations at these time points were
1,104 ± 228 ng/ml (IV) and 1,331 ± 297 ng/ml (oral). The mean $C_{\text{max}}$ for pentoxifylline IV was 10,672 ± 2,689 ng/ml, compared with a $C_{\text{max}}$ of 1,734 ± 871 ng/ml for the PO route (Table 1). AUC was 310,274 ± 99,069 ng/ml/min for the IV route and 655,405 ± 372,402 ng/ml/min for the PO route. Mean residence time was 196 ± 293 minutes for IV administration and 612 ± 355 minutes for PO administration. The rate of elimination ($K_{\text{el}}$) following IV administration (0.022 ± 0.019 minutes) was significantly ($P = .04$) greater than that following PO administration (0.004 ± 0.004* minutes).
than following PO administration (0.004 ± 0.004 minutes), suggesting the rate of absorption is slower than the rate of absorption (i.e., a “flip-flop” model). The corresponding elimination half-life was 164 ± 227 minutes (IV) and 404 ± 267 minutes (PO). The PO bioavailability of pentoxifylline was 50% ± 26%.

Both metabolites M1 and M5 were detected at most sampling times in all dogs (Figure 2). However, data for M1 were not sufficient following both routes of administration to allow modeling in all dogs, thus precluding adequate description behavior in several dogs. M5 appeared to be the dominant metabolite as compared with M1 for both routes based on AUC. \( C_{\text{max}} \) and \( C_0 \) were significantly \((P = .0001)\) greater for M5 than for pentoxifylline or M1. Following IV administration, the mean residence time (MRT) was 170 ± 106 minutes for M5 compared with 0.71 ± 0.3 minutes for M1. Following PO administration, the MRT for M5 was 374 ± 110 minutes compared with 266 ± 295 minutes for M1. The relative bioavailability for M5 (AUC following IV administration versus PO and corrected for dose) was greater than M1 (108% ± 31% versus 37% ± 17%).

**DISCUSSION**

Pentoxifylline is known for both its immunologic and hematologic properties. The drug exerts its immunologic and wound-healing properties by decreasing inflammatory mediators (e.g., tumor necrosis factor and interleukin 1) and by stimulating collagenase. In humans, pentoxifylline inhibits platelet aggregation, decreases fibrinogen levels, and deforms red blood cells, all which appear to facilitate blood flow. M1 and M5 are responsible for the hematologic effects observed with pentoxifylline. Previous studies have not addressed whether these hematologic effects occur in dogs treated with pentoxifylline. In the present study (contrary to what occurs in humans), neither platelet aggregation nor fibrinogen concentrations differed following administration of pentoxifylline either IV or PO. This may reflect a species difference, or the lack of hematologic response also may reflect failure to achieve therapeutic con-
centrations. However, in humans, the mean therapeutic pentoxifylline drug concentration appears to be 1,000 ng/ml. In the present study, peak concentrations following PO administration were 1,734 ± 871 ng/ml and IV concentrations exceeded 10 times the therapeutic range in humans. Thus, failure to impact hemostasis in dogs does not appear to reflect failure to achieve therapeutic concentrations. However, the difference between human and dog studies might also reflect a difference between single and multiple dosing. It is possible that multiple dosing is required to achieve a physiologic steady state. Hematologic studies should be repeated in dogs following multiple dosing over several weeks at doses that deliver the drug at 30 mg/kg before ruling out a lack of hematologic effects of pentoxifylline in dogs.

A previous study in dogs detected M1 but not M5. In contrast (but in accordance with previous human studies) M5 was present in the dogs treated in the present study. In fact, as was previously shown in humans, M5 was present in significantly (P = .0001) greater concentrations (both C max and AUC) than either pentoxifylline or M1 when comparing data for pentoxifylline given by the same route of administration. However, following PO administration, the presence of M5 was over 300 fold greater than that of M1, as evidenced by relative bioavailability (AUC for the PO route versus AUC for the IV route corrected for dosage differences).

Differences in AUC for M5 following PO versus IV administration may reflect first-pass metabolism of pentoxifylline to M5. The predominance of M5 over M1 may reflect, in part, the metabolic path of M5 formation in that both pentoxifylline and M1 can be metabolized to M5. Because M1 is formed in red blood cells, and the present study measured compounds in plasma, concentrations of this metabolite may have been underrepresented.

A number of pharmacokinetic parameters also differed in this study compared with findings in previous human or dog studies. In humans, pentoxifylline bioavailability is 19.4% ± 12.7%, whereas a previous study in dogs reported a bioavailability of 30.4% ± 3.2%. These two results are lower than the bioavailability measured in the present study (50% ± 26%). The PO dose used in this study was twice that used by other investigators. Thus, differences in bioavailability may reflect, in part, an individual variation, which may be exacerbated with the use of a dose that differed when compared to previous studies. Furthermore, the higher PO dose used in this study may have allowed concentrations to be detected for a longer period, thus increasing the AUC. Food was not likely to be a factor in differences in PO bioavailability because food does not appear to interfere with the absorption of pentoxifylline in dogs as it does in humans. Additionally, feeding protocols in relationship to dosing were similar between this study and previous studies in dogs.

The elimination half-life for pentoxifylline was markedly shorter following IV versus PO administration, implying a “flip-flop” model. The terminal component of the plasma drug concentration versus time curve reflects absorption of pentoxifylline rather than removal of the drug from the body. Thus, the elimination half-life more appropriately reflects a disappearance in half-life. This is to be expected of a sustained-release product. Elimination half-life was among the parameters that differed among species (human versus dog) and studies. In the present study, elimination half-life in dogs (404 ± 267 minutes) was more similar to that reported in humans (204 minutes) than that previously reported in dogs (22.1 ± 2.9 minutes) using the same PO preparation as that used in the present study. Half-life is impacted by both clearance (directly) and volume of distribution (inversely), and differences in these
two parameters in this study compared with findings in previous studies account for some of the difference in half-life. Clearance of pentoxifylline was less in the present study (28 ± 7 ng/ml/min) than that reported for a previous study (37 ± 4 ng/ml/min) and VDss was four fold higher (4 L/kg) than that in other investigations (1 L/kg). Differences in pentoxifylline clearance between the present study and the previous dog study were not as profound as were differences in half-life. Reasons for differences in clearance may simply reflect animal variability, including breed differences (hound breed in the current study versus mixed breed in the previous report) and the small number of animals studied. Gender differences were probably not a factor because the previous study was also conducted with female dogs. However, because only female animals were studied, information in this study does not necessarily accurately represent disposition in male dogs. Additional studies that involve male dogs ultimately may be indicated.

Differences in half-life between this study and the previous dog study also may reflect differences in the dose and its impact on the detection of drug concentrations (lower LOQ for the present study). A lower LOQ in the present study (30 versus 50 ng/ml) allowed detection of lower drug concentration for a longer period of time. As such, the terminal portion of the curve, upon which half-life is based, might have been more accurately represented in the present study.

Based on an elimination half-life of approximately 6 hours, an 8-hour dosing interval might be necessary for pentoxifylline to maintain a clinical response. Therapeutic drug concentrations above 1,000 ng/ml were detected in the present study for 170 ± 24 minutes for IV and 510 ± 85 minutes for PO administration. This finding suggests a 12-hour dosing regimen may be appropriate. However, a longer interval may be possible if metabolites contribute significantly to efficacy, or if response is maintained in the absence of detectable drug in plasma.

**CONCLUSIONS**

Pentoxifylline appears to be well absorbed, well tolerated, and readily bioavailable. A higher pentoxifylline PO dose (30 mg/kg) than previously reported was not associated with any adverse effects in any dog. Since therapeutic pentoxifylline concentrations were present for 8.5 hours after PO drug administration, a 12-hour dosing regimen seems reasonable. Similar to reports in humans, M5 appears to be the predominant metabolite and may be important in the ability of the parent drug to exert its therapeutic effects. Although metabolites appear to play a major role in the disposition of pentoxifylline in dogs, their role in the hematologic effects need to be elucidated. This study did not identify hematologic effects of pentoxifylline or its metabolites following a single dosing. The beneficial properties of pentoxifylline in dogs may be due to its immunologic and not hematologic properties.

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