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Pharmacokinetics of the cytochrome P-450 substrates phenytoin, theophylline, and diazepam in healthy Greyhound dogs

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Abstract

The purpose of this study was to determine the pharmacokinetics of phenytoin, theophylline, and diazepam in six healthy Greyhound dogs. Additionally the pharmacokinetics of the diazepam metabolites oxazepam and nordiazepam after diazepam administration were determined. Phenytoin sodium (12 mg/kg), aminophylline (10 mg/kg), and diazepam (0.5 mg/kg) were administered IV on separate occasions and blood obtained at predetermined time points for the quantification of plasma drug concentrations by fluorescence polarization immunoassay (phenytoin, theophylline) or mass spectrometry (diazepam, oxazepam, nordiazepam). The terminal half-life was 4.9, 9.2, and 1.0 hours, respectively for phenytoin, theophylline, and diazepam, and 6.2 and 2.4 hours for oxazepam and nordiazepam after IV diazepam. The clearance was of 2.37, 0.935, and 27.9 mL/min/kg respectively for phenytoin, theophylline, and diazepam. The C_{MAX} was 44.7 and 305.2 ng/mL for oxazepam and nordiazepam, respectively, after diazepam administration. Temazepam was not detected above 5 ng/mL in any sample after IV diazepam.

Introduction

The pharmacokinetics of some cytochrome P-450 (CYP) substrates appear to be different in Greyhounds, but the extent of the differences has not been fully evaluated. One of the first indications that Greyhounds may metabolize some drugs slower was the slow recovery from thiopental anesthesia. Pharmacokinetic studies indicated thiopental concentrations persisted above 10 µg/mL mean plasma concentrations in Greyhounds for 8 hours compared to 0.75 hours for mixed-breed dogs (Sams, et al, 1985). The persistence of mean thiopental concentrations above 10 µg/mL in Greyhounds treated with phenobarbital, a CYP inducer, was less than 2 hours suggesting deficient CYP activity was responsible for the persistence of thiopental plasma concentrations and prolonged recovery in Greyhounds (Sams & Muir, 1988).

Antipyrine is considered a nonspecific marker for CYP activity. A pharmacokinetic study compared the disposition of antipyrine in Greyhounds and Beagles and determined the plasma clearance in Greyhounds (8.33 mL/min/kg) was significantly slower than Beagles (13.42 mL/min/kg) and the half-life was significantly longer in Greyhounds (1.09 h) compared to Beagles (0.55 h) (KuKanich et al, 2007). A separate study in Coonhounds determined the plasma clearance of antipyrine (6.2 mL/min/kg) was more similar to Greyhounds than Beagles (Gurley et al, 1997), but comparisons between different studies may not be accurate. Therefore it remains unclear if Beagles are ultra-metabolizers or Greyhounds are poor metabolizers of antipyrine relative to other dog breeds.

The clearance of IV propofol in Greyhounds (54.0 ± 10 mL/min/kg) was significantly slower than mixed-breed dogs (114.8 ± 50 mL/min/kg), but the half-life in Greyhounds (175.68 ± 179 min) was similar to mixed-breed dogs (122.04 ± 55.6 min) (Zoran et al, 1993). Subsequent *in vitro* studies demonstrated microsomal activity of Greyhounds was significantly slower than Beagle microsomes, but Greyhound microsomes were not different from mixed-breed dog microsomes (Hay Kraus, et al, 2000). Propofol was identified primarily as a CYP2B11 substrate and chloramphenicol, a CYP2B11 inhibitor, inhibits propofol metabolism (Mandsager et al, 1995; Hay Kraus, et al, 2000).

The plasma concentrations of oral chloramphenicol were compared in Greyhounds and similar sized mongrel dogs (Watson, 1974). There was no significant difference in the plasma concentrations at any time point suggesting similar pharmacokinetics.

Phenytoin metabolism is inhibited by chloramphenicol and induced by phenobarbital in dogs suggesting it is metabolized, at least in part by CYP2B11 in dogs (Cucinell, et al 1963; Sanders et al, 1979). Theophylline clearance is inhibited by enrofloxacin in dogs and fluoroquinolones are inhibitors of CYP1A suggesting theophylline is at least in part a CYP1A substrate (Intorre et al, 1995; Regmi et al 2005). Diazepam is metabolized to nordiazepam by CYP2B11 and nordiazepam is metabolized to oxazepam by CYP3A12 in dogs while diazepam is metabolized to temazepam by CYP3A12 and temazepam is metabolized to oxazepam by CYP2B11 in dogs (Shou et al, 2003; Lu et al, 2005).

No reports were available describing the pharmacokinetics of phenytoin, theophylline, or diazepam in Greyhound dogs. In addition, no reports describing the metabolic profile of diazepam in Greyhounds were available. The purpose of this study was to describe the pharmacokinetics the apparent CYP2B11 substrates phenytoin and diazepam, and a non-CYP2B11 substrate, theophylline, in Greyhound dogs.

Material and Methods

Animals

Six healthy Greyhounds (3 male and 3 female), 3–5 years of age weighing 28.5 – 43.1 kg were included in the study. The Institutional Animal Care and Use Committee at Kansas State University approved the study.

Drug administration and sample collection

Drugs were administered in a non-randomized design, theophylline as aminophylline, 10 mg/kg IV equivalent to 7.88 mg/kg theophylline, (aminophylline 25 mg/mL, Hospira, Inc, Lake Forest, IL, USA), then sodium phenytoin, 12 mg/kg equivalent to 11 mg/kg phenytoin IV (sodium phenytoin 50 mg/mL, Hospira, Inc, Lake Forest, IL, USA), and then diazepam, 0.5 mg/kg IV (5 mg/mL, Hospira, Inc, Lake Forest, IL, USA), with at least 3 weeks between each treatment. Drugs were administered through an indwelling IV catheter in the cephalic vein while blood samples for the determination of plasma drug concentrations were obtained from a catheter in the jugular vein. The cephalic catheter was flushed with 10 mL 0.9% sodium chloride after drug injection. Blood samples, 9 mL per time point, were obtained prior to drug administration and at 10, 20, and 30 minutes and at 1, 2, 4, 8, 12, 24, 48, and 56 hours after aminophylline administration. Blood samples, 9 mL per time point, were obtained prior to drug administration and at 10, 20, and 30 minutes, and at 1, 2, 4, 8, 12, 24, and 32 hours after sodium phenytoin administration. Blood samples, 9 mL per time point, were obtained prior to drug administration and at 5, 10, 20, 30, and 45 minutes and at 1, 2, 4, 6, 8, 12 and 24 hours after diazepam administration. The whole blood was placed into tubes containing lithium heparin, immediately placed on ice after collection, centrifuged at $3000 \times$

g for 20 minutes within 2 hours of collection, and the plasma was separated and stored frozen at -70°C prior to drug analysis.

Plasma drug concentrations of theophylline and phenytoin

Plasma concentrations of theophylline and phenytoin were measured with a commercially available fluorescence polarization immunoassay (FPIA) (TDx, Abbott Laboratories, Abbott Park, IL, USA) according to manufacturer directions. Standard curves and quality control (QC) samples were constructed by adding known amounts of theophylline (Acros Organics USA, Morris Plains, NJ, USA) including 0 and 2.5–40 $\mu\text{g/mL}$ (in duplicate) or phenytoin (Acros Organics USA, Morris Plains, NJ, USA) to untreated canine plasma including 0 and 2.5–40 $\mu\text{g/mL}$ (in duplicate) for each analyte. Standard curves were accepted if the predicted values were within 15% of the actual values for the standard curves and at least 2/3 QC samples (low, medium, high) were within 15% for each run per manufacturer recommendations. The limit of quantification was 0.82 $\mu\text{g/mL}$ for theophylline as determined by the manufacturer of the instrument and reagents and is defined as the lowest measurable concentration which can be distinguished from 0 with a 95% confidence. The limit of quantification for phenytoin was 0.5 $\mu\text{g/mL}$ for phenytoin as determined by the manufacturer of the instrument and reagents and is defined as the lowest measurable concentration which can be distinguished from 0 with a 95% confidence.

High Pressure Liquid Chromatography determination of theophylline

The correlation of the theophylline plasma drug concentrations determined by FPIA and high pressure liquid chromatography (HPLC) with ultraviolet (UV) detection was determined. Ten theophylline plasma samples ranging from 1.29 to 11.06 $\mu\text{g/mL}$ (determined by FPIA) were additionally assayed by HPLC to assess the correlation of the assays. Plasma samples were diluted 1:1 with 0.1% trifluoroacetic acid and then the plasma water was separated with an ultrafiltration device (Amicon Centifree, 10,000 MW cutoff, Millipore Corp., Bedford, MA, USA) by centrifugation at $2000 \times g$ for 30 minutes. The plasma standards were constructed by adding known amounts of theophylline (Acros Organics USA, Morris Plains, NJ, USA) to untreated canine plasma. The plasma standard curve was linear from 1 – 20 $\mu\text{g/mL}$ and was accepted if the coefficient of determination (R^2) was at least 0.99. The injection volume was 0.05 mL. The mobile phase consisted of 10% acetonitrile and 90% TFA (0.1%) at a flow rate of 1 mL/min and a C-18, 4.6 μm , 150 mm $\times$ 4.6 mm column (Zorbax XDB C-18, Agilent technologies, Wilmington, DE) achieved separation at ambient temperature. The UV absorption was monitored at 270 nm. The accuracy and coefficient of variation (CV) of the theophylline HPLC assay were determined on replicates of 3 plasma QC samples for each of the following concentrations: 1, 5, and 10 $\mu\text{g/mL}$. The accuracy and CV were $99 \pm 3\%$ of the actual concentration and 0.8%, respectively. The correlation of measured FPIA and HPLC plasma theophylline concentrations was determined by linear regression with no weighting using computer software (SigmaPlot, Systat Software, Richmond, CA, USA).

High Pressure Liquid Chromatography determination of phenytoin

Plasma concentrations of phenytoin were determined by HPLC after using solid phase extraction (SPE) (Oasis HLB, Waters Corporation, Milford, MA, USA). Ten phenytoin plasma samples ranging from 0.53 to 13.18 $\mu\text{g/mL}$ (determined by FPIA) were additionally assayed by HPLC to assess correlation of the assays. The SPE were conditioned with 1 mL methanol, followed by 1 mL deionized water. Plasma, 0.5 mL, was loaded to the SPE and then the SPE were washed with 1 mL 5% methanol. Samples were eluted with 1 mL methanol, which was evaporated to dryness and subsequently reconstituted with 0.2 mL 15% methanol. The reconstituted samples were centrifuged at $5000 \times g$ for 5 minutes to separate any particulates and the supernatant transferred to an injection vial. The injection

volume was 0.05 mL. The plasma standards were constructed by adding known amounts of phenytoin (Acros Organics USA, Morris Plains, NJ, USA) to untreated canine plasma from 0.2 – 20 µg/mL. The standard curve was accepted if the predicted concentrations were within 15% of the actual concentration and the R^2 was at least 0.99. The mobile phase consisted of 50% TFA (0.02%) and 50% acetonitrile at a flow rate of 1 mL/min. A C-18, 4.6 µm × 150 mm × 4.6 mm column (Zorbax XDB C-18, Agilent technologies, Wilmington, DE) achieved separation at ambient temperature. The UV absorbance was quantified at 220 nm. The accuracy and coefficient of variation (CV) of the phenytoin HPLC assay were determined on replicates of 3 plasma QC samples for each of the following concentrations: 0.2, 1, and 10 µg/mL. The accuracy and CV were 98 ± 4% of the actual concentration and 4%, respectively. The correlation of measured FPIA and HPLC plasma phenytoin concentrations was determined by linear regression with no weighting using computer software (SigmaPlot, Systat Software, Richmond, CA, USA).

Liquid Chromatography / Mass Spectrometry method of analysis for diazepam and metabolites

The plasma concentrations of diazepam (m/z 285.1→193.3), oxazepam (m/z 287.07→241.30), nordiazepam (m/z 271.1→140.1), and temazepam (m/z 301.1→255.2) were determined with oxazepam d5 (m/z 292.0→246.00) as an internal standard (IS) by liquid chromatography (Shimadzu Prominence, Shimadzu Scientific Instruments, Columbia, MD, USA) and mass spectrometry (API 2000, Applied Biosystems, Foster City, CA, USA). The reference standards for diazepam, diazepam metabolites, and IS were from the same source (Cerilliant Corporation, Round Rock, TX, USA). Plasma standard curves were made in untreated canine plasma from 5 – 2500 ng/mL for diazepam, oxazepam, nordiazepam, and temazepam. Standard curves were accepted if the predicted concentrations were within 15% of the actual concentration and the correlation coefficient was at least 0.99. Plasma standards and incurred samples were extracted in an identical manner. Plasma was extracted using solid phase extraction cartridges (SPE) (Varian Bond Elut C18, Varian Inc. Palo Alto, CA). Plasma, 0.5 mL, was added to 0.1 mL IS (500 ng/mL) and 0.5 mL 0.1 M sodium borate buffer and vortexed for 5 seconds. The SPE were conditioned with 1 mL methanol, followed by 1 mL deionized water, then the plasma mixture (1.1 mL) was loaded, followed by rinsing the SPE with 1 mL 5% methanol. The sample was eluted with 1 mL methanol. The eluate was evaporated under an air stream at 40° C for 30 minutes. The samples were reconstituted with 0.2 mL 50% methanol and 25 µL was the injection volume. The mobile phase consisted of A: acetonitrile and B: 0.1% formic acid at a flow rate of 0.4 mL/min. A gradient starting at 75% B from 0 – 1 min to 45% B from 1 – 3 minutes which was held until 5 minutes and then back to 75% B at 6 minutes with a total run time of 7 minutes. Separation was achieved with a C18 column (Supelco Discovery C18, 50 mm x 2.1 mm x 5 µm, Sigma-Aldrich, St. Louis, MO, USA) maintained at 40° C. The accuracy of the assay for diazepam was 99 ± 6% and the CV was 5% determined on replicates of 5 each at 5, 250, and 1000 ng/mL. The accuracy of the assay for oxazepam was 99 ± 5% and the CV was 5% determined on replicates of 5 each at 5, 250, and 1000 ng/mL. The accuracy of the assay for nordiazepam was 99 ± 5% and the CV was 6% determined on replicates of 5 each at 5, 250, and 1000 ng/mL. The accuracy of the assay for temazepam was 99 ± 6% and the CV was 6% determined on replicates of 5 each at 5, 250, and 1000 ng/mL. The limit of quantification was 5 ng/mL for diazepam and metabolites defined as the lowest concentration on the standard curve with predicted concentrations within 15% of the actual concentration.

Pharmacokinetic Analysis

Pharmacokinetic analyses were performed with computer software (WinNonlin 5.2, Pharsight Corporation, Mountain View, CA, USA) using noncompartmental methods. The

parameters included the area under the curve from time 0 to infinity (AUC) using the linear trapezoidal rule, percent of the AUC extrapolated to infinity ($AUC_{\text{extrapolated}}$), plasma clearance (Cl), apparent volume of distribution at steady state (V_{ss}), apparent volume of distribution (area method) (V_z), first-order terminal rate constant (λ_z), terminal half-life ($T_{1/2}$), and mean residence time extrapolated to infinity (MRT). The maximum plasma concentration (C_{MAX}) of oxazepam and nordiazepam and the time to maximum plasma concentration (T_{MAX}) of oxazepam and nordiazepam after diazepam administration were determined directly from the data. The plasma clearance per bioavailability (Cl/F) of oxazepam and nordiazepam after IV diazepam and the V_z per bioavailability (V_z/F) of oxazepam and nordiazepam after IV diazepam were also determined.

Results

The FPIA for theophylline plasma concentrations was well correlated ($R^2=0.9963$) with the HPLC method as determined on 10 plasma samples ranging from 1.29 to 11.06 $\mu\text{g/mL}$. The correlation of phenytoin concentrations determined by FPIA and HPLC was also very good ($R^2=0.9627$) on 10 samples from 0.53 – 13.18 $\mu\text{g/mL}$.

The pharmacokinetic parameters for theophylline are presented in Table 1 and the plasma profile in Figure 1. The mean V_z was 0.747 L/kg with a range of 0.684 – 0.817 L/kg. The mean Cl was 0.935 mL/min/kg with a range of 0.831 – 1.223 mL/min/kg. The mean $T_{1/2}$ was 9.2 hours with a range of 7.0 – 11.3 h.

The pharmacokinetic parameters for phenytoin are presented in Table 2 and the plasma profile in Figure 2. The mean V_z was 1.004 L/kg with a range of 0.745 – 1.192 L/kg. The mean Cl was 2.37 mL/min/kg with a range of 1.79 – 3.26 mL/min/kg. The mean $T_{1/2}$ was 4.9 hours with a range of 3.8 – 6.8 h.

The pharmacokinetic parameters for diazepam are presented in Table 3 and the plasma profile in Figure 3. The mean V_z was 2.448 L/kg with a range of 1.974 – 3.083 L/kg. The mean Cl was 27.9 mL/min/kg with a range of 22.4 – 44.9 mL/min/kg. The mean $T_{1/2}$ was 1.0 hours with a range of 0.5 – 1.4 h.

Temazepam was not detected above the analytical LOQ, 5 ng/mL. The mean C_{MAX} of oxazepam was 44.7 ng/mL with a range of 31.7 – 58.1 ng/mL at a mean T_{MAX} of 0.70 h (Table 4, Figure 3). The mean C_{MAX} of nordiazepam was 305.2 ng/mL with a range of 249.0 – 342.0 ng/mL at a mean T_{MAX} of 0.36 h (Table 5, Figure 3).

Discussion

The purpose of this study was to assess the pharmacokinetics of two different drugs that apparently are metabolized by CYP2B11 in Greyhounds, which are reported to have decreased elimination of some CYP2B11 substrates. The pharmacokinetics of theophylline, a CYP1A substrate in dogs, was also assessed to determine the pharmacokinetics of a CYP substrate which apparently utilizes a different CYP isoform for metabolism.

Despite the availability of pharmacokinetic studies in other dog breeds for theophylline, phenytoin, and diazepam direct comparisons with the pharmacokinetic parameters reported in this paper are inappropriate. Differences in study design including dose, rate of dose administration, times of sample collection, assay methodology, environmental effects, animal weight, animal body condition, animal age, random inter-animal and intra-animal variability make direct comparisons inappropriate. Therefore the results of this study will provide a basis for future studies comparing the pharmacokinetics of these drugs in different dog breeds.

Previous studies have reported the pharmacokinetics of theophylline after IV aminophylline administration to dogs (McKiernan, et al, 1981; Shiu, et al, 1989; Bach, et al 2004). The pharmacokinetics of 11 mg/kg aminophylline, equivalent to 8.6 mg/kg theophylline, IV to non-Greyhound, non-Beagle dogs resulted in a mean $T_{1/2}$, Cl, and V_z of 8.4 hr, 0.780 mL/min/kg, and 0.546 L/kg (Bach, et al 2004). In contrast a study in mixed-breed dogs administered aminophylline 12 mg/kg, equivalent to 9.4 mg/kg, theophylline IV, resulting in a mean theophylline $T_{1/2}$ and V_z of 5.7 hr and 0.8244 L/kg (McKiernan, et al, 1981). Beagles administered 100 mg aminophylline IV had a $T_{1/2}$, Cl, and V_d of 4.5 hr, 2.1 mL/min/kg, and 0.834 L/kg (Shiu, et al, 1989). It is unclear as to why the latter studies were apparently different than the other studies. The McKiernan, et al (1981) and Shiu et al (1989) studies used an HPLC method whereas the Bach et al (2004) study used an FPIA method. However results of the correlation of FPIA and HPLC in this study were very good suggesting the potential differences may not have been due to the assay. Any potential differences may just be due to random study variability, differences in study design or pharmacokinetic analysis, therefore direct comparisons may not be inappropriate. The pharmacokinetics of theophylline in Greyhounds included a mean $T_{1/2}$, Cl, and V_z of 9.2 hr, 0.935 mL/min/kg, and 0.747 L/kg respectively and are within the range of values previously reported in non-Greyhound dogs. A direct comparison of the pharmacokinetics of theophylline in multiple breeds of dogs were not performed, therefore true differences or similarities cannot be assessed.

The pharmacokinetics of phenytoin administered IV to dogs as a single dose have been previously reported (Cucinell et al, 1963; Sanders, et al, 1979; Frey & Löscher, 1980). The pharmacokinetics of IV phenytoin, 15 mg/kg, in Beagles reported a mean $T_{1/2}$, Cl, and V_z of 3.1 hr, 4.68 mL/min/kg, and 1.092 L/kg, respectively (Sanders, et al, 1979). The $T_{1/2}$ of phenytoin, 50 mg/kg IV, in mongrel dogs was 6.7 h, but other pharmacokinetic parameters were not reported (Cucinell et al, 1963). The $T_{1/2}$, Cl, and V_z of phenytoin, 10 mg/kg IV, to non-Beagle, non-Greyhound dogs, were 4.4 hr, 2.8 mL/min/kg, and 1.0 L/kg, respectively (Frey & Löscher, 1980). The pharmacokinetics of phenytoin in Greyhounds included a mean $T_{1/2}$, Cl, and V_z of 4.9 hr, 2.37 mL/min/kg, and 1.004 L/kg. The $T_{1/2}$, Cl, and V_z for the Greyhounds in this study were within the range of reported values in mongrel and other dog breeds. The Beagle study reported a shorter half-life and more rapid clearance, but it is unclear if the difference is a true difference in the pharmacokinetics or due to study design or random variability. A direct comparison of the pharmacokinetics of phenytoin were not performed, therefore true differences or similarities cannot be assessed.

The pharmacokinetics of IV diazepam have been previously reported in dogs (Löscher & Frey, 1981; Papich & Alcorn, 1995; Musulin et al 2011). The range of the terminal half-life of diazepam in non-Greyhound dogs has been 0.25 – 5.5 hours (Löscher & Frey, 1981; Papich & Alcorn, 1995; Musulin et al 2011). The reported plasma clearance of diazepam in non-Greyhound dogs ranged from 11.5 – 60 mL/min/kg (Papich & Alcorn, 1995; Musulin et al 2011). The reported range of diazepam V_z in non-Greyhound dogs is 1.4 – 3.5 L/kg. The pharmacokinetics of IV diazepam in Greyhound dogs administered 0.5 mg/kg resulted in a mean $T_{1/2}$, Cl, and V_z of 1.0 hr, 27.9 mL/min/kg, and 2.448 L/kg, respectively. The pharmacokinetics of IV diazepam in Greyhounds is within the range previously reported in non-Greyhound dogs. The large ranges of diazepam pharmacokinetic parameters and different study conditions make interpretations of similarity or differences difficult and such interpretations may not be accurate.

The range of oxazepam C_{MAX} , dose normalized to 0.5 mg/kg diazepam, ranged from approximately 20 – 39 ng/mL in non-Greyhound dogs (Löscher & Frey, 1981; Papich & Alcorn, 1995; Musulin et al 2011) compared to 44.7 ng/mL in Greyhounds. The AUC (to infinity) of oxazepam in non-Greyhound dogs after IV diazepam, normalized to 0.5 mg/kg

ranged from 400 – 421 hr*ng/mL (Löscher & Frey, 1981; Musulin et al 2011) compared to 405.2 hr*ng/mL in Greyhound dogs. The terminal half-lives of oxazepam ranged from 3.5 – 6.5 hours after IV diazepam in non-Greyhound dogs (Löscher & Frey, 1981; Papich & Alcorn, 1995; Musulin et al 2011) compared to 6.2 hours in Greyhounds. The pharmacokinetics of oxazepam after IV diazepam administration in Greyhounds are within ranges previously reported in non-Greyhound dogs. A direct comparison of the pharmacokinetics of oxazepam after diazepam administration in multiple breeds of dogs were not performed, therefore true differences or similarities cannot be assessed.

The C_{MAX} of nordiazepam, normalized to 0.5 mg/kg IV diazepam in non-Greyhound dogs has a reported range of 183 to 350 ng/mL (Löscher & Frey, 1981; Papich & Alcorn, 1995; Musulin et al 2011) compared to 305.2 hr*ng/mL in Greyhounds. The AUC (to infinity) of nordiazepam after IV diazepam, normalized to 0.5 mg/kg, in non-Greyhound dogs has a reported range of 1047 – 1625 hr*ng/mL compared to 976 hr*ng/mL in Greyhounds. The terminal half-life of nordiazepam, after diazepam administration, in non-Greyhound dogs has a reported range of 2.2 – 6.7 hours compared to 2.4 hours in Greyhounds. The pharmacokinetics of nordiazepam after IV diazepam administration in Greyhounds are within ranges previously reported in non-Greyhound dogs. A direct comparison of the pharmacokinetics of nordiazepam after diazepam administration in multiple breeds of dogs were not performed, therefore true differences or similarities cannot be assessed.

Diazepam is metabolized to nordiazepam by CYP2B11 and nordiazepam is metabolized to oxazepam by CYP3A12 in dogs while diazepam is metabolized to temazepam by CYP3A12 and temazepam is metabolized to oxazepam by CYP2B11 in dogs (Shou et al, 2003; Lu et al, 2005). The high concentrations of nordiazepam in Greyhounds are suggestive that Greyhounds were able to efficiently metabolize diazepam by a mechanism previously identified as CYP2B11 in dogs. However the specific metabolizing enzymes in these Greyhounds were not identified and the rapid elimination of diazepam and formation of nordiazepam may have occurred by CYP2B11 or a different enzyme. Temazepam was not detected above the LOQ of the analytical assay (5 ng/mL) after 0.5 mg/kg diazepam IV to Greyhounds. This is suggestive that either temazepam is not a major metabolite in Greyhounds or it is rapidly metabolized to another metabolite (oxazepam) prior to reaching systemic circulation.

In conclusion the pharmacokinetics of theophylline, and the purported CYP2B11 substrates phenytoin and diazepam in Greyhounds are within previously reported ranges in non-Greyhound dogs. Further studies are needed directly comparing the pharmacokinetics of these drugs in Greyhounds and non-Greyhound dogs in order to determine if the pharmacokinetics are truly similar or different or if any similarities or differences are due to study design, analytical methods, or study environment.

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Abbreviations

AUC	the area under the curve from time 0 to infinity using the linear trapezoidal rule
AUC_{extrapolated}	percent of the AUC extrapolated to infinity

Cl	plasma clearance
Cl/F	plasma clearance per bioavailability of diazepam metabolites after diazepam administration
V_{ss}	apparent volume of distribution at steady state
V_z	apparent volume of distribution (area method)
V_z/F	V _z per bioavailability of diazepam metabolites after diazepam administration
λ_z	first-order terminal rate constant
T_{1/2}	terminal half-life
MRT	mean residence time extrapolated to infinity
C_{MAX}	maximum plasma concentration
T_{MAX}	time to maximum plasma concentration
LOQ	Lower limit of analytical quantification

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Figure 1.
Plasma concentrations (mean and standard deviation) of theophylline in 6 healthy Greyhounds after IV aminophylline (10 mg/kg equivalent to 7.88 mg/kg theophylline base).

Figure 2.
Plasma concentrations (mean and standard deviation) of phenytoin in 6 healthy Greyhounds after IV sodium phenytoin (12 mg/kg equivalent to 11 mg/kg phenytoin base).

Figure 3.
Plasma concentrations (mean and standard deviation) of diazepam, oxazepam, and nordiazepam in 6 healthy Greyhound dogs after IV diazepam (0.5 mg/kg)

Table 1

Theophylline pharmacokinetic parameters in 6 healthy Greyhounds after IV administration of aminophylline (10 mg/kg equivalent to 7.88 mg/kg theophylline).

Parameter	Units	Mean	Minimum	Median	Maximum
AUC _{extrapolated}	%	10.9	8.9	10.5	14.1
AUC	hr*μg/mL	140.4	107.4	151.5	158.1
C ₀	μg/mL	10.9	9.3	11.0	12.0
Cl	mL/min/kg	0.935	0.831	0.868	1.223
T _{1/2}	hr	9.2	7.0	9.4	11.3
λ _z	1/hr	0.0751	0.0612	0.0737	0.0996
MRT	hr	12.9	9.6	13.0	15.8
V _{ss}	L/kg	0.721	0.650	0.717	0.792
V _z	L/kg	0.747	0.684	0.742	0.817

Table 2

Phenytoin pharmacokinetic parameters in 6 healthy Greyhounds after IV administration of sodium phenytoin (12 mg/kg equivalent to 11 mg/kg phenytoin).

Parameter	Units	Mean	Minimum	Median	Maximum
AUC _{extrapolated}	%	3.84	1.11	4.11	18.46
AUC	hr*µg/mL	77.4	56.2	79.0	102.6
C ₀	µg/mL	16.1	7.6	15.4	41.7
Cl	mL/min/kg	2.37	1.79	2.38	3.26
T _{1/2}	hr	4.9	3.8	4.9	6.8
λ _z	1/hr	0.142	0.103	0.141	0.185
MRT	hr	6.74	5.20	6.79	9.22
V _{ss}	L/kg	0.96	0.72	0.99	1.14
V _z	L/kg	1.004	0.745	1.036	1.192

Table 3

Diazepam pharmacokinetic parameters in 6 healthy Greyhounds after IV administration of diazepam (0.5 mg/kg).

Parameter	Units	Mean	Minimum	Median	Maximum
AUC _{extrapolated}	%	2.9	2.3	2.9	3.9
AUC	hr*ng/mL	298.7	185.8	331.6	371.5
C ₀	ng/mL	1193.7	855.7	1181.3	1775.4
Cl	mL/min/kg	27.9	22.4	25.1	44.9
T _{1/2}	hr	1.0	0.5	1.1	1.4
λ_z	1/hr	0.684	0.503	0.617	1.363
MRT	hr	0.54	0.38	0.59	0.64
V _{ss}	L/kg	0.908	0.656	0.962	1.043
V _z	L/kg	2.448	1.974	2.359	3.083

Table 4

Oxazepam pharmacokinetic parameters in 6 healthy Greyhounds after IV administration of diazepam (0.5 mg/kg IV).

Parameter	Units	Mean	Minimum	Median	Maximum
AUC _{extrapolated}	%	27.0	15.3	25.1	55.6
AUC	hr*ng/mL	405.4	262.5	401.2	599.2
Cl/F	mL/min/kg	20.6	13.9	21.3	31.8
C _{MAX}	ng/mL	44.7	31.7	44.9	58.1
T _{1/2}	hr	6.2	3.9	5.5	12.7
λ_z	l/hr	0.112	0.055	0.125	0.178
MRT	hr	9.4	6.0	8.3	19.2
T _{MAX}	hr	0.79	0.33	1.00	1.00
V _Z /F	L/kg	11.063	8.307	11.034	15.283

Table 5

Nordiazepam pharmacokinetic parameters in 6 healthy Greyhounds after IV administration of diazepam (0.5 mg/kg IV).

Parameter	Units	Mean	Minimum	Median	Maximum
AUC _{extrapolated}	%	3.6	1.7	3.5	7.7
AUC	hr*ng/mL	976.8	624.8	1000.5	1418.3
Cl/F	mL/min/kg	8.53	5.88	8.36	13.34
C _{MAX}	ng/mL	305.2	249.0	312.0	342.0
T _{1/2}	hr	2.4	1.5	2.4	4.1
λ_z	1/hr	0.290	0.170	0.295	0.453
MRT	hr	3.4	2.2	3.3	5.4
T _{MAX}	hr	0.36	0.33	0.33	0.50
V _Z /F	L/kg	1.766	1.560	1.713	2.077