Pharmacokinetics of zonisamide following rectal administration to healthy dogs

Jennifer R. Michaels DVM
Amy J. Hodshon DVM
William B. Thomas DVM
Dawn M. Boothe DVM, PhD
Lindsay Williams DVM

Received December 12, 2015
Accepted March 16, 2016

From the Department of Small Animal Clinical Sciences, College of Veterinary Medicine, University of Tennessee, Knoxville, TN 37996 (Michaels, Hodshon, Thomas, Williams); and Department of Anatomy, Physiology and Pharmacology, College of Veterinary Medicine, Auburn University, Auburn, AL 36849 (Boothe). Dr. Williams' present address is Carolina Veterinary Specialists, 1600 Hanes Mall Blvd, Winston-Salem, NC 27103.

Address correspondence to Dr. Michaels (jmicha12@utk.edu).

OBJECTIVE
To evaluate the pharmacokinetics of zonisamide following rectal administration of 20 or 30 mg/kg suspended in sterile water or polyethylene glycol (PEG) to healthy dogs and determine whether either dose resulted in plasma zonisamide concentrations within the recommended therapeutic target range (10 to 40 μg/mL).

ANIMALS
8 healthy mixed-breed dogs.

PROCEDURES
Each dog received each of 2 doses (20 or 30 mg/kg) of zonisamide suspended in each of 2 delivery substrates (sterile water or PEG) in a randomized crossover study with a 7-day washout period between phases. A blood sample was collected from each dog immediately before and at predetermined times for 48 hours after zonisamide administration. Plasma zonisamide concentrations were determined by high-performance liquid chromatography, and data were analyzed with a noncompartmental model.

RESULTS
Mean maximum plasma concentration, time to maximum plasma concentration, mean residence time, and elimination half-life did not differ significantly among the 4 treatments. The mean maximum plasma concentration for all 4 treatments was less than the therapeutic target range. The mean ± SD area under the concentration-time curve for the 30 mg/kg-in-water treatment (391.94 ± 237.00 h•μg/mL) was significantly greater than that for the 20 mg/kg-in-water (146.19 ± 66.27 h•μg/mL) and 20 mg/kg-in-PEG (87.09 ± 96.87 h•μg/mL) treatments.

CONCLUSIONS AND CLINICAL RELEVANCE
Results indicated that rectal administration of zonisamide at doses of 20 and 30 mg/kg failed to achieve plasma zonisamide concentrations within the recommended therapeutic target range. Therefore, rectal administration of zonisamide cannot be recommended as a suitable alternative to oral administration. (*Am J Vet Res* 2016;77:1374–1380)

Seizures are the most common neurologic disorder of dogs, and idiopathic epilepsy is the most common cause of chronic, recurring seizures.[1] If untreated, seizures can result in substantial illness and death. Aside from the physical ramifications of seizures in affected dogs, recurrent seizures are a source of emotional stress and financial strain for their owners. Consequently, the goal for the treatment of affected dogs is to reduce seizure severity and frequency. There are multiple treatment options; however, not all patients respond to the most commonly used drugs, and many of those drugs have undesirable adverse effects.

ABBREVIATIONS

$AUC_{0-\infty}$	Area under the concentration-time curve from time 0 to infinity
C_{max}	Maximum plasma concentration
HPLC	High-performance liquid chromatography
$MRT_{0-\infty}$	Mean residence time from time 0 to infinity
PEG	Polyethylene glycol
$t_{1/2}$	Elimination half-life
t_{max}	Time to maximum plasma concentration

Zonisamide is a sulfonamide anticonvulsant with several likely mechanisms of action including modulation of the dopaminergic and serotonergic systems, binding to γ-aminobutyric acid chloride channels, inhibition of glutamate release, and blockage of t-type calcium and voltage-gated sodium channels.[2–4] Zonisamide is most commonly used as an adjunctive maintenance therapy for dogs with refractory idiopathic epilepsy. Results of 2 prospective open-label studies[2,3] indicate that the addition of zonisamide to the treatment regimen (ie, phenobarbital or potassium bromide) of dogs with recurrent seizures decreases seizure frequency, and for some dogs, the addition of zonisamide to the treatment regimen made it possible to decrease the dosage of the first-line antiseizure medication, thus reducing the adverse effects associated with that medication and decreasing the treatment cost for the owners. Oral administration of zonisamide has also been used as the first-line treatment or monotherapy for dogs with idiopathic epilepsy. In another prospective open-label study,[5] 6 of 10

dogs with idiopathic epilepsy that were treated with zonisamide (5 to 10 mg/kg, PO, q 12 h) monotherapy had a ≥ 50% decrease in the monthly frequency of seizures.

Adverse effects associated with zonisamide administration in dogs are typically minor and transient and include mild sedation, ataxia, and vomiting.[1,2,5] Zonisamide is metabolized primarily by hepatic microsomal enzymes, and a mild increase in alkaline phosphatase activity is possible with chronic administration.[2,6] Erythema multiforme[7] and apparent idiosyncratic hepatic necrosis[8,9] have been reported in 1 and 2 dogs, respectively, and were most likely attributable to sulfonamide hypersensitivity. Zonisamide administration was associated with renal tubular acidosis in 1 dog,[10] which might have been the result of zonisamide-induced inhibition of carbonic anhydrase.[4,10]

In the United States, zonisamide is currently commercially available only as an encapsulated powder for oral administration. Investigators of previous pharmacokinetic studies[4,11–13] produced and used an injectable zonisamide solution. However, because that formulation is not commercially available, zonisamide powder has anecdotally been administered per rectum to dogs that are too sedate or ill from adverse effects of other drugs or neurologic or systemic disease to safely swallow medication. This practice was extrapolated and adopted on the basis of data that suggest therapeutic target plasma concentrations of zonisamide are achieved in rodents following administration of zonisamide suppositories.[12] The published literature contains only sparse pharmacological data regarding rectal administration of zonisamide to dogs. Results of 1 study[14] indicate that the plasma zonisamide concentration of dogs following rectal administration of zonisamide (10 mg/kg) dissolved in water or as a suppository after being compounded with PEG was significantly lower than that achieved following oral administration of the same dose of the drug. The empirical dose of zonisamide (30 mg/kg) that is commonly used for rectal administration was extrapolated on the basis of the results of that study[14] and the study[12] performed in rodents. Objective data regarding the pharmacokinetics of zonisamide following rectal administration to dogs are lacking; therefore, the appropriate dose of zonisamide for rectal administration to dogs is unknown.

The purpose of the study reported here was to determine the pharmacokinetics of zonisamide in healthy dogs following rectal administration of each of 2 doses (20 and 30 mg/kg) suspended in sterile water or PEG. Our goals were to use that information to determine the optimal drug delivery substrate (water or PEG) and recommend an appropriate dose of zonisamide for rectal administration to dogs. We hypothesized that rectal administration of commercially available zonisamide powder suspended in water or PEG at doses of 20 and 30 mg/kg would result in plasma zonisamide concentrations within the recommended therapeutic target range (10 to 40 µg/mL) and that the C_{max} following administration of zonisamide suspended in PEG would be greater than that following administration of zonisamide suspended in sterile water. Finally, we compared the pharmacokinetics of zonisamide following rectal administration as determined in this study to those following oral administration as determined in previous studies[4,11,14] to determine whether rectal administration might be an appropriate substitute route of zonisamide administration for dogs in which oral administration of medications is contraindicated.

Materials and Methods

Animals

Eight (6 female and 2 male) mixed-breed dogs with body weight ranging from 10.5 to 30.8 kg and age ranging from 2 to 13 years were used for the study. Prior to study enrollment, each dog was determined to be healthy on the basis of results of a physical examination, CBC, and serum biochemical analysis. The dogs were a part of a university research colony and were housed in individual runs at that institution's laboratory animal care facility. All research protocols were approved by the Institutional Animal Care and Use Committee of the University of Tennessee.

Experimental protocol

The study had a crossover design with 4 phases. A computerized randomization generator was used to assign each dog to 1 of 4 groups (2 dogs/group) in a random manner. During each phase, the dogs in each group received 1 dose per rectum of 1 of 4 zonisamide-delivery substrate combinations (20 mg/kg in water, 30 mg/kg in water, 20 mg/kg in PEG, or 30 mg/kg in PEG). A blood sample was collected from each dog immediately before and at predetermined times for 48 hours after zonisamide administration. There was a 7-day washout period between phases, and the process was repeated until all dogs had received all 4 zonisamide-delivery substrate combinations. This resulted in 32 unique dog and zonisamide-delivery substrate combinations (trials).

Zonisamide suspension

Zonisamide powder was extracted from commercially available enteric capsules.[a] The powder was then mixed with water or PEG to create 2 zonisamide suspensions, each with a zonisamide concentration of 100 mg/mL. Prior to study initiation, it was determined that a suspension with 100 mg of zonisamide/mL allowed for adequate suspension of the zonisamide powder in the respective liquids while minimizing the volume required for administration to each dog. The zonisamide powder could not be adequately maintained in suspension in either water or PEG at concentrations > 100 mg/mL. The zonisamide suspensions were prepared with sterile equipment in a laminar-flow hood to minimize con-

tamination. In addition, personnel preparing the suspensions wore goggles, masks, and gloves to further reduce the risk of contamination and to protect themselves against zonisamide exposure. The suspensions were prepared < 2 hours before rectal administration. Briefly, for each phase, the volume of each suspension type (zonisamide-water and zonisamide-PEG) required for the dogs assigned to receive the 20 and 30 mg/kg doses was calculated, then that volume of water and PEG was added to separate sterile bottles. The amount of zonisamide powder required to create a suspension with a drug concentration of 100 mg/mL was added to each bottle. The contents of each bottle were vigorously mixed to suspend the powder.

Drug administration

For each of the 4 phases, food but not water was withheld from all dogs for 12 hours prior to zonisamide administration. For each dog, a gloved index finger was used to digitally evacuate all fecal material from the rectum immediately before zonisamide administration. The zonisamide suspension was administered through a 10-cm red rubber catheter that was inserted approximately 2 to 4 cm (depending on the size of the dog) into the rectum. The catheter was flushed with approximately 4 mL of air immediately after the zonisamide was administered to ensure that the dog received the entire dose. The catheter was removed from the rectum and the anus was held closed for approximately 1 minute to minimize drug expulsion. Dogs were monitored for 48 hours after zonisamide administration for suspension retention (ie, defecation) and adverse effects.

Sample collection

For each dog, a blood sample (approximately 2 mL) was collected via jugular venipuncture directly into sterile tubes[b] containing lithium heparin with a vacuum blood collection system immediately before (0 hours) and at 1, 3, 6, 9, 12, 18, 24, and 48 hours after zonisamide administration. The duration of that observation period represented at least 3 plasma elimination half-lives for zonisamide following oral administration.[4] The blood samples were centrifuged immediately after collection, and the plasma was harvested from each sample and stored in polypropylene vials at –80°C until analysis (storage time, 1.5 to 6 months).

Determination of plasma zonisamide concentration

The zonisamide concentration in each plasma sample was determined by use of reverse-phase HPLC with UV detection at 245 nm as described.[14] Briefly, the plasma samples were thawed and vortexed. Then, 100 µL of each sample was placed in a separate tube followed by 400 µL of acetonitrile. Each tube was capped, vortexed, and centrifuged for 10 minutes at 13,000 × *g*. Next, 100 µL of the resulting supernatant was transferred to an HPLC vial. A 25-µL aliquot of each supernatant sample was injected into the HPLC system for analysis. The compounds were separated on a reverse-phase C18 column (150 × 4.6 mm, 5 µm) protected by a C8 precolumn (5 µm). The mobile phase consisted of purified distilled water and acetonitrile (65:35) with a flow rate of 1 mL/min. The limit of quantitation was 1 µg/mL. The target range for the therapeutic plasma concentration of zonisamide (10 to 40 µg/mL) chosen for the dogs of this study was the same as that used for human patients.[1-4]

Data analysis

Pharmacokinetic parameters were calculated by use of standard noncompartmental methods with commercially available linear-regression software.[c] Parameters of interest included $AUC_{0-\infty}$, C_{max}, t_{max}, $MRT_{0-\infty}$, and $t_{1/2}$. The $AUC_{0-\infty}$ was calculated by use of the linear trapezoidal rule. All parameters were reported as the mean ± SD except the $t_{1/2}$, which was reported as the harmonic mean ± pseudo-SD.

A 2-way ANOVA was used to compare pharmacokinetic parameters among the 4 zonisamide treatments, in which the response variable was a specific pharmacokinetic parameter and the independent variables were treatment (20 mg/kg in water, 30 mg/kg in water, 20 mg/kg in PEG, or 30 mg/kg in PEG) and whether the dog defecated within 1 hour after zonisamide administration (yes or no). All ANOVA analyses were performed with commercially available software,[d] and values of $P < 0.05$ were considered significant.

Results

The volume of zonisamide suspension administered per dog ranged from 1.9 to 6.2 mL for the 20 mg/kg-in-water treatment, 3.2 to 8.8 mL for the 30 mg/kg-in-water treatment, 2.1 to 6.2 mL for the 20 mg/kg-in-PEG treatment, and 3.2 to 9.2 mL for the 30 mg/kg-in-PEG treatment. The dog defecated within 1 hour after zonisamide administration in 9 (4 trials when the zonisamide dose administered was 20 mg/kg and 5 trials when the zonisamide dose administered was 30 mg/kg) of the 32 trials (unique dog and zonisamide-delivery substrate combinations). One dog vomited once approximately 6 hours after receiving the 30 mg/kg-in-PEG treatment. No other adverse effects were observed.

Data for 5 (2 trials for the 30 mg/kg-in-water treatment, 2 trials for the 20 mg/kg-in-PEG treatment, and 1 trial for the 30 mg/kg-in-PEG treatment) of the 32 trials were excluded from the analysis because the plasma zonisamide concentration was less than the limit of quantitation (1 µg/mL) for the HPLC assay used at all sampling times. In all 5 of the excluded trials, the dog defecated within 1 hour after zonisamide administration. After those 5 trials were excluded, none of the pharmacokinetic parameters of interest were significantly associated with whether

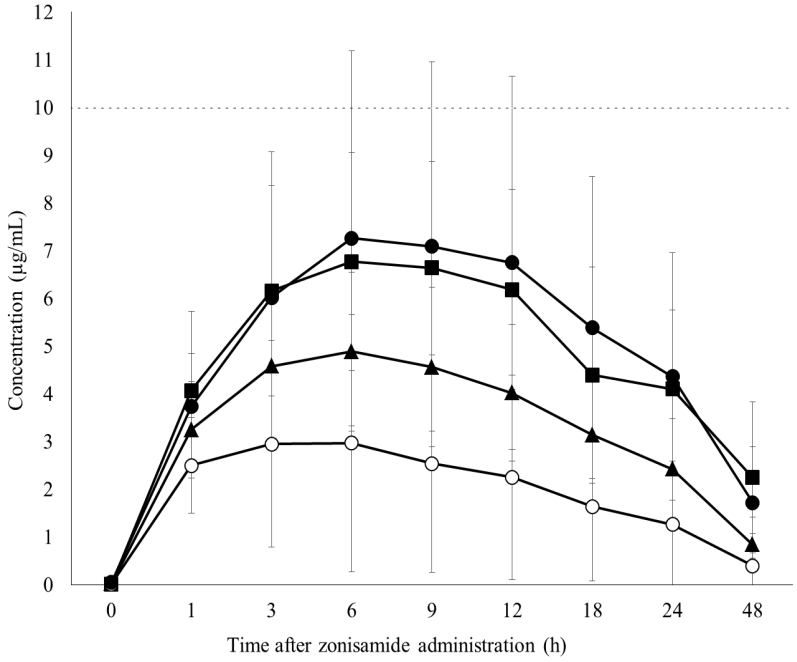

Figure 1—Mean ± SD plasma zonisamide concentration over time following rectal administration of the drug to 8 healthy mixed-breed dogs. Each dog received each of 2 doses (20 or 30 mg/kg) of zonisamide suspended in each of 2 delivery substrates (sterile water or PEG) in a randomized crossover study with a 7-day washout period between phases. Thus, each dog received each of 4 treatments (20 mg/kg in water [black triangles], 30 mg/kg in water [black squares], 20 mg in PEG [white circles], and 30 mg/kg in-PEG [black circles]). Data were obtained for 32 trials (unique dog and zonisamide-delivery substrate combinations); however, data for 5 trials were excluded from the analysis because the plasma zonisamide concentration was < 1 µg/mL (the HPLC limit of quantitation) at all sampling times. Eight dogs contributed to the mean for the 20 mg/kg-in-water treatment, 6 dogs contributed to the mean for the 30 mg/kg-in-water treatment, 6 dogs contributed to the mean for the 20 mg/kg-in-PEG treatment, and 7 dogs contributed to the mean for the 30 mg/kg-in-PEG treatment. The dashed line represents the lower limit of the currently recommended target range (10 to 40 µg/mL) for the therapeutic plasma concentration of zonisamide.

the dog defecated within 1 hour after zonisamide administration.

The mean ± SD plasma zonisamide concentrations over time for each of the treatments were depicted graphically **(Figure 1).** Plasma zonisamide concentrations > 10 µg/mL (the lower limit of the therapeutic target range) were achieved in only 2 trials; the 30 mg/kg-in-PEG treatment was administered in both of those trials. The plasma zonisamide concentration was > 10 µg/mL at 6, 9, and 12 hours after drug administration in 1 trial and at 3, 6, 9, and 12 hours after drug administration in the other trial.

The pharmacokinetic parameters of interest for each of the 4 treatments were summarized **(Table 1)**. The C_{max}, t_{max}, $MRT_{0-\infty}$, and $t_{1/2}$ did not differ significantly among the treatments. The mean $AUC_{0-\infty}$ for the 30 mg/kg-in-water treatment was significantly greater than that for the 20 mg/kg-in-water ($P = 0.037$) and 20 mg/kg-in-PEG ($P = 0.013$) treatments.

Discussion

Results of the present study indicated that rectal administration of zonisamide at doses of 20 and 30 mg/kg to healthy dogs failed to achieve plasma zonisamide concentrations within the recommended therapeutic target range (10 to 40 µg/mL) for the drug. Therefore, rectal administration

Table 1—Plasma pharmacokinetic parameters for zonisamide following rectal administration of the drug at doses of 20 or 30 mg/kg suspended in water or PEG to 8 healthy mixed-breed dogs.

	Zonisamide treatment			
Parameter	20 mg/kg in water (n = 8)	30 mg/kg in water (n = 6)	20 mg/kg in PEG (n = 6)	30 mg/kg in PEG (n = 7)
$AUC_{0-\infty}$ (h•µg/mL)	146.19 ± 66.27[a]	391.94 ± 237.0[b]	87.09 ± 96.867[a]	261.09 ± 168.018[a,b]
C_{max} (µg/mL)	4.9253 ± 1.77	7.862 ± 0.9813	3.289 ± 2.81	7.698 ± 4.10
t_{max} (h)	6.0 ± 1.604	6.60 ± 3.286	2.17 ± 2.041	5.00 ± 3.830
$MRT_{0-\infty}$ (h)	24.52 ± 4.966	53.04 ± 48.497	23.14 ± 5.703	28.36 ± 6.261
$t_{1/2}$ (h)	15.26 ± 3.24	23.51 ± 11.56	14.96 ± 4.13	16.78 ± 3.27

Each dog received each of 2 doses (20 or 30 mg/kg) of zonisamide suspended in each of 2 delivery substrates (sterile water or PEG) in a randomized crossover study with a 7-day washout period between phases. Thus, data for 32 trials (unique dog and zonisamide-delivery substrate combinations) were obtained. Data for 5 trials (2 trials for the 30 mg/kg-in-water treatment, 2 trials for the 20 mg/kg-in-PEG treatment, and 1 trial for the 30 mg/kg-in-PEG treatment) were excluded from the standard noncompartmental pharmacokinetic analysis because the plasma zonisamide concentration was < 1 µg/mL (the HPLC limit of quantitation) at all sampling times. All parameters were reported as the mean ± SD except the $t_{1/2}$, which was reported as the harmonic mean ± pseudo-SD.

[a,b]Values with different superscript letters differ significantly ($P < 0.05$).

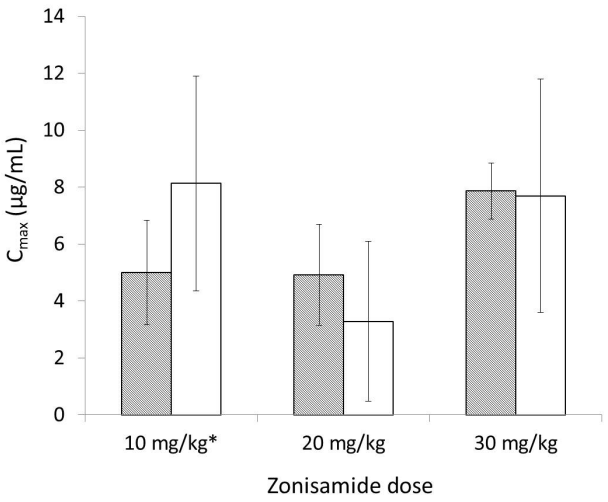

Figure 2—Mean ± SD C_{max} for zonisamide in healthy dogs following rectal administration of the drug at doses of 10, 20, and 30 mg/kg in combination with water (striped bars) or PEG (white bars) as determined in the present study (20 and 30 mg/kg doses) or a previous study[14] (10 mg/kg). *Zonisamide was administered as a suspension for all treatments except the 10 mg/kg dose with PEG, which was administered as a suppository. Eight dogs contributed to the means for the 10 mg/kg treatments.

of zonisamide is not a suitable alternative to oral administration of the drug. Additionally, results suggested that the plasma zonisamide concentration was not significantly affected by either the drug dose or delivery substrate (water or PEG).

Currently, the therapeutic target range for plasma zonisamide concentration (10 to 40 µg/mL) most frequently recommended for dogs is extrapolated from that used in human medicine.[1-4] Only 2 dogs achieved a plasma zonisamide concentration > 10 µg/mL at any time during the present study; both dogs achieved that concentration when administered the 30 mg/kg-in-PEG treatment. For those 2 dogs, the plasma zonisamide concentration was within the therapeutic target range beginning at 3 and 6 hours after drug administration and had fallen below the therapeutic target range by 18 hours after drug administration. The highest plasma zonisamide concentration (13.45 µg/mL) recorded was for a dog 6 hours after it was administered the 30 mg/kg-in-PEG treatment. The mean C_{max} for each of the 4 rectally administered zonisamide treatments of the present study (Table 1) was much lower than the mean C_{max} (14.4 µg/mL) achieved following oral administration of 1 dose (10 mg/kg) of zonisamide to dogs in another study.[4]

The currently recommended dose of zonisamide for oral administration to dogs is 5 to 10 mg/kg.[1] We used higher doses (20 and 30 mg/kg) of zonisamide for rectal administration in the present study on the basis of results of other studies[12,14] that suggest such doses would be necessary to achieve plasma zonisamide concentrations within the therapeutic target range following rectal administration of the drug. We chose to administer zonisamide in a suspension rather than in a suppository because rectal administration of zonisamide is most likely to be considered for hospitalized dogs in which oral drug administration is temporarily contraindicated because of profound sedation or abnormal mentation, an absent gag reflex, or persistent vomiting, conditions that cannot be anticipated. Presumably, such dogs would require rectal administration of zonisamide for immediate or short-term treatment, and there may not be sufficient time for suppository preparation. The time required to compound a suppository precludes the use of that drug delivery method when immediate drug administration is necessary. However, a zonisamide suspension can be created by any veterinary professional on an as-needed basis with little specialized equipment.

We chose to suspend zonisamide in water because of convenience and in PEG on the basis of data that suggested PEG might be a more effective medium for zonisamide delivery than water.[12,14] In another study,[12] zonisamide was rectally administered to rodents in a suppository after formulation with Witepsol (a lipophilic base) or PEG. Although therapeutic blood concentrations of zonisamide were achieved following both treatments, zonisamide absorption was more rapid from the PEG suppository than from the Witepsol suppository.[12] Moreover, zonisamide was administered to rodents in that study,[12] and the metabolism of drugs in rodents often differs substantially from that in other species. Zonisamide is metabolized in the liver by cytochrome p450 isoenzymes, acetylation, and glucuronidation.[4,8] Dogs have unique hepatic metabolism and lack genes that encode for acetylation. Thus, metabolism data for drugs that require acetylation obtained by the use of rodents or primates might not accurately translate to dogs.[4,8]

Dogs that were rectally administered zonisamide (10 mg/kg) as a suspension in water or as a suppository after formulation with PEG also failed to achieve therapeutic target plasma concentrations of zonisamide.[14] The investigators of that study[14] postulated that rectal administration of a higher dose of zonisamide might result in target plasma concentrations of the drug; however, the results of the present study refute that. In the present study, although the mean $AUC_{0-\infty}$ for the 30 mg/kg-in-water treatment was significantly greater than that for the 20 mg/kg-in-water and 20 mg/kg-in-PEG treatments, there were no other data to support a significant association between zonisamide dose and plasma zonisamide concentration. Additionally, when we plotted the mean ± SD C_{max} for the 4 zonisamide treatments evaluated in the present study alongside the mean ± SD C_{max} for 2 zonisamide treatments (10 mg of zonisamide/kg rectally administered as a suspension in water or as a suppository after formulation with PEG) evaluated in that other study,[14] it was obvious that, despite differences in study methods, a 3-fold increase in the dose

of zonisamide administered per rectum had little effect on the C_{max} **(Figure 2)**. Thus, increasing the dose of zonisamide administered per rectum in dogs is unlikely to result in plasma zonisamide concentrations within the therapeutic target range.

It is important to note several differences in methods between the present study and the study[14] in which dogs were rectally administered 10 mg of zonisamide/kg that make comparison of the pharmacokinetic data between the 2 studies unpredictable. In the other study,[14] zonisamide formulated with PEG was administered as a suppository rather than as a suspension as it was in the present study. Also, following administration of the zonisamide-water suspension in that other study,[14] investigators flushed the catheters with water to ensure that the entire dose was delivered. In the present study, we flushed the catheters with air following administration of the zonisamide suspensions. It is possible that flushing the catheter with air allowed zonisamide powder within the residual suspension in the catheter lumen to settle such that the entire drug dose was not actually delivered to the dogs, which would have affected the results of the present study. However, flushing the catheters with water would have changed the zonisamide concentration of the suspensions delivered to the dogs, and the addition of the flush water to the zonisamide-PEG suspensions might have changed the properties of the drug delivery substrate, which might have affected drug absorption and hence the pharmacokinetic parameters. In the present study, the zonisamide powder appeared to be well-suspended in the delivery substrates prior to administration; therefore, we believe that flushing the catheters with air immediately after drug administration resulted in minimal loss of zonisamide powder from the residual suspension in the catheter lumen.

The presence of feces in the rectum can impair absorption of rectally administered drugs.[15] In the present study, the rectum of each dog was manually evacuated of feces in an attempt to minimize fecal impairment of zonisamide absorption and reduce the chance of stimulating defecation during or following drug administration. Despite that precaution, in 9 of 32 trials, the dog defecated within 1 hour after zonisamide administration, and suspension medium or white powder was visually evident in the feces of 4 of those trials. For 5 of the 9 trials in which the dog defecated within 1 hour after zonisamide administration, the plasma zonisamide concentration was less than the HPLC limit of quantitation at all sampling times. For the remaining 4 trials, the plasma zonisamide concentration was greater than the HPLC limit of quantitation for at least 5 of the 9 sampling times. The plasma zonisamide concentration was greater than the HPLC limit of quantitation for at least 5 of the 9 sampling times for all trials in which the dog did not defecate within 1 hour after zonisamide administration. Although the risk for defecation might have been decreased by the administration of an enema prior to zonisamide administration, we chose to not perform enemas in an attempt to better approximate the conditions in which we believe rectal administration of zonisamide will be used clinically.

One challenge for the rectal administration of zonisamide is achieving a high concentration of the drug in suspension so that the volume necessary for administration can be minimized. Prior to initiation of the present study, we determined that 100 mg of zonisamide/mL was the maximum concentration that would allow for adequate suspension of the zonisamide powder in the delivery substrate. Had evidence suggested the existence of a positive association between zonisamide dose and plasma zonisamide concentration, it is unlikely that a sufficient dose of zonisamide could be rectally administered as a suspension to achieve therapeutic plasma concentrations of the drug because the volume required would distend the rectum and stimulate defecation. In the present study, there did not appear to be a correlation between the volume of zonisamide suspension administered per body weight and defecation within 1 hour after drug administration. The frequency of defecation within 1 hour after zonisamide administration was fairly high in the present study even though the volume of the zonisamide suspension administered was comparable to or less than that rectally administered to dogs of other studies.[14,15] It is possible that many factors (eg, rectal wall compliance, existing disease or irritation of the rectal mucosa, temperature or chemical makeup of the compound administered, and degree of distention in other areas of the gastrointestinal tract) other than volume may affect enteric nervous system reflexes and contribute to the likelihood of defecation after rectal stimulation.

Many epileptic dogs for which rectal administration of zonisamide might be considered are already being treated with a combination of zonisamide and phenobarbital. Phenobarbital increases the rate of clearance for zonisamide, resulting in a lower C_{max} for zonisamide, which necessitates the administration of a higher dose of zonisamide to maintain the plasma zonisamide concentration within the therapeutic target range.[13] Thus, the dose of zonisamide (regardless of route of administration) administered to any dog concurrently receiving phenobarbital will need to be higher than that administered to dogs not receiving phenobarbital to achieve therapeutic plasma zonisamide concentrations.

The present study had several limitations. The random crossover design, number of dogs evaluated, and blood sampling times used in this study were similar to those of other studies[4,11,13,14] conducted to determine the pharmacokinetics of zonisamide following oral and rectal administration. Nevertheless, the small sample number of dogs may have limited our ability to identify statistically significant effects. Additionally, the complete data from 5 of the 32 trials

performed were excluded from the analysis because the plasma zonisamide concentration was below the HPLC limit of quantitation at all sampling times, which effectively further decreased the study population and limited our power to identify significant differences. The dog defecated within 1 hour after zonisamide administration in all 5 of the excluded trials. Thus, it is likely that the effect of defecation on the pharmacokinetic parameters was underestimated. Although exclusion of those 5 trials decreased the number of trials evaluated for 3 of the 4 treatments, we do not believe it affected the mean C_{max} or our conclusions regarding the lack of a dose effect on plasma zonisamide concentration.

We did not measure the final concentration of zonisamide in the prepared suspensions prior to administration. It is possible that the actual concentration of zonisamide in the administered suspensions differed from the intended concentration of 100 mg/mL. However, we believe that such a discrepancy was minimal given that the zonisamide powder was extracted from commercially available capsules and added directly to the required volumes of both water and PEG calculated to achieve a suspension with the intended concentration. The methods used to prepare the zonisamide suspensions in this study were designed to mimic techniques that would likely be used in a clinical setting.

In the present study, rectal administration of zonisamide at doses of 20 or 30 mg/kg suspended in water or PEG to healthy dogs failed to achieve plasma zonisamide concentrations within the therapeutic target range (10 to 40 µg/mL), despite the fact that preparation and administration of the suspensions was quick and convenient. Results also indicated that there was no association between zonisamide dose and plasma concentrations of the drug. Therefore, increasing the dose of zonisamide administered per rectum is unlikely to achieve therapeutic plasma concentrations of the drug, and administration of zonisamide cannot be recommended as a suitable alternative to oral administration for dogs that require treatment with that drug.

Acknowledgments

Supported by grants from the American College of Veterinary Internal Medicine and the University of Tennessee College of Veterinary Medicine Companion Animal Fund.

Presented as an oral presentation at the 2015 American College of Veterinary Internal Medicine Forum, Indianapolis, June 2015.

The authors thank Tamberlyn Moyer for technical assistance and Xiaocun Sun for assistance with statistical analysis.

Footnotes

a. Zonisamide 100-mg capsules, Mylan, Canonsburg, Penn.
b. Monoject lithium heparin tubes, Fisher Scientific, Itasca, Ill.
c. WinNonLin, Pharsight Corp, Mountain View, Calif.
d. SSPS Statistics for Windows, verion 2.0, IBM Corp, Armonk, NY.

References

1. Thomas WB. Seizures and narcolepsy. In: Dewey CW, ed. *A practical guide to canine and feline neurology*. 2nd ed. Ames, Iowa: Wiley-Blackwell, 2008;237–259.
2. Dewey CW, Guiliano R, Boothe DM, et al. Zonisamide therapy for refractory idiopathic epilepsy in dogs. *J Am Anim Hosp Assoc* 2004;40:285–291.
3. Von Klopmann T, Rambeck B, Tipold A. Prospective study of zonisamide therapy for refractory idiopathic epilepsy in dogs. *J Small Anim Pract* 2007;48:134–138.
4. Boothe DM, Perkins J. Disposition and safety of zonisamide after intravenous and oral single dose and oral multiple dosing in normal hound dogs. *J Vet Pharmacol Ther* 2008;31:544–553.
5. Chung JY, Hwang CY, Chae JS, et al. Zonisamide monotherapy for idiopathic epilepsy in dogs. *N Z Vet J* 2012;60:357–359.
6. Walker RM, DiFonzo CJ, Barsoum NJ, et al. Chronic toxicity of the anticonvulsant zonisamide in Beagle dogs. *Fundam Appl Toxicol* 1988;11:333–342.
7. Ackerman AL, Frank LA, McEntee MF, et al. Erythema multiforme associated with zonisamide in a dog. *Vet Dermatol* 2015;26:391–392.
8. Miller ML, Center SA, Randolph JF, et al. Apparent acute idiosyncratic hepatic necrosis associated with zonisamide administration in a dog. *J Vet Intern Med* 2011;25:1156–1160.
9. Schwartz M, Munana KR, Olby NJ. Possible drug-induced hepatopathy in a dog receiving zonisamide monotherapy for treatment of cryptogenic epilepsy. *J Vet Med Sci* 2011;73:1505–1508.
10. Cook AK, Allen AK, Espinosa D, et al. Renal tubular acidosis associated with zonisamide therapy in a dog. *J Vet Intern Med* 2011;25:1454–1457.
11. Fukunaga K, Saito M, Muto M, et al. Steady-state pharmacokinetics of zonisamide in plasma, whole blood and erythrocytes in dogs. *J Vet Pharmacol Ther* 2010;33:103–106.
12. Nagatomi A, Mishima M, Tsuzuki O, et al. Utility of a rectal suppository containing the antiepileptic drug zonisamide. *Biol Pharm Bull* 1997;20:892–896.
13. Orito K, Saito M, Fukunaga K, et al. Pharmacokinetics of zonisamide and drug interaction with phenobarbital in dogs. *J Vet Pharmacol Ther* 2008;31:259–264.
14. Brewer D, Cerda-Gonzalez S, Dewey CW, et al. Pharmacokinetics of single-dose rectal zonisamide administration in normal dogs. *J Vet Intern Med* 2015;29:603–606.
15. Peters RK, Schubert T, et al. Levetiracetam rectal administration in healthy dogs. *J Vet Intern Med* 2014;28:504–509.