Journal of Veterinary Internal Medicine
Open Access

J Vet Intern Med 2014;29:603–606

Pharmacokinetics of Single-Dose Rectal Zonisamide Administration in Normal Dogs

D.M. Brewer, S. Cerda-Gonzalez, C.W. Dewey, D. Boothe, and K. Van Horne

Background: Few medications are available for parental administration to animals with seizures. Rectal administration of medications is often used if the animal cannot be administered oral medications.

Hypothesis/Objectives: To determine the pharmacokinetic differences in zonisamide when administered rectally in either of 2 vehicles and PO to dogs.

Animals: Eight healthy research dogs.

Methods: Randomized cross-over design. Zonisamide, 10 mg/kg, was administered rectally in polyethylene glycol (PEG-R), rectally in water (H_2O-R), and as an oral capsule. Plasma zonisamide concentrations were measured until 72 hours after administration. Zonisamide was quantitated by HPLC and plasma concentration versus time curve data was analyzed by using noncompartmental modeling.

Results: Mean maximum plasma zonisamide concentrations (μg/mL) were significantly higher after oral administration (11.56 ± 4.04) compared to H_2O-R (5.00 ± 1.83) (P = .004). Disappearance half-life (hours) and mean time to maximum concentration (hours) were not significantly different between methods of administration. Mean relative bioavailability of PEG-R (85 ± 69%) was significantly higher than that of H_2O-R (53 ± 37%) (P = .039). Dogs tolerated all dosing forms with no evidence of adverse effects.

Conclusions and Clinical Importance: The vehicle in which zonisamide is dissolved influences rectal bioavailability, with PEG preferred to H_2O-R. Because of the prolonged time to maximum concentration, rectal administration of zonisamide should not be used to treat status epilepticus in dogs. A dose higher than what was used in this study might be necessary, if currently recommended minimum therapeutic concentrations (10 μg/mL) are to be achieved with a single-dose administration.

Key words: Bioavailability; Epilepsy; Polyethylene glycol; Seizure; Status epilepticus.

Seizures are one of the most common neurologic disorders of dogs.[1,2] A subset of epileptic dogs experience cluster seizures (two or more seizures within a 24-hour period), or status epilepticus (repeated seizure activity with a failure to return to normal within 30 minutes) during their lifetime.[3] Such dogs routinely receive multiple doses of medications with sedative properties, anesthetic properties, or both, such as diazepam, midazolam, phenobarbital, pentobarbital, or propofol within a short period of time, in an attempt to halt their seizure activity. In many of these cases, by the time that short-term control has been achieved, the animal is too sedated to receive oral medications. Similarly, dogs with seizure activity coupled with an altered mentation secondary to a forebrain lesion, or with abnormal pharyngeal function, esophageal function, or both, because of multifocal brain involvement might be unable to receive oral medications without risk of aspiration. Despite these limitations, in both of these situations longer acting antiepileptic medications must be initiated once immediate seizure control has been achieved. Treatment options for such cases include intravenous administration of phenobarbital and sodium bromide. However, often animals are already being treated with 1 or both of these medications at therapeutic drug levels. It is estimated that 25–30% of epileptic dogs will be refractory to appropriate doses of these anticonvulsants and additional options for instituting maintenance treatment are needed.[1,4,5]

Zonisamide (1,2-benzisoxazole-3-methanesulfonamide) is a safe and effective antiepileptic drug in dogs.[6–8] Therapeutic plasma levels of zonisamide in dogs range from 10 to 40 μg/mL.[6,7] Currently, zonisamide is approved only as an oral formulation in humans. However, when suspended in either Witepsol or polyethylene glycol (PEG)[10–12], and administered as a rectal suppository to rats, the bioavailability of zonisamide approximated 100%, with the PEG formulation being most rapidly absorbed.[13] The purpose of this study was to determine the pharmacokinetics of 2 formulations of zonisamide when administered rectally as a single dose,

Abbreviations:

AUC	area under the curve
C_{max}	maximum concentration
H_2O-R	water-rectal
HPLC	high-performance liquid chromatography
MRT	mean resistance time
PEG-R	polyethylene glycol-rectal
$T_{1/2}$	half-life
T_{max}	time to maximum concentration

From the Department of Clinical Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY (Brewer, Cerda-Gonzalez, Dewey, Van Horne); and the Department of Anatomy, Physiology and Pharmacology, College of Veterinary Medicine, Auburn University, Auburn, AL (Boothe).

This work was performed at a research facility associated with the Cornell University Hospital for Animals.

These results were presented in abstract form in the Journal for Veterinary Emergency and Critical Care 2008; 18(4): 410.

Corresponding author: S. Cerda-Gonzalez; T6 002B Vet Res Tower, College of Veterinary Medicine, Cornell University, Ithaca, NY 14850; e-mail: sc224@cornell.edu.

Submitted June 12, 2014; Revised November 7, 2014; Accepted December 10, 2014.

DOI: 10.1111/jvim.12540

to determine plasma concentrations achieved after rectal administration, to compare these to the recommended therapeutic concentrations, and to assess the safety of zonisamide when administered rectally as a single dose to normal dogs.

Material and Methods

Eight healthy intact adult dogs weighing 26–39 kg were used for this study. Evidence of health was based on physical examination, neurologic examination, bloodwork (complete blood count and chemistry panel), and urinalysis. All dogs received 3 separate doses of approximately 10 mg/kg of zonisamide in the following forms: as a rectal suppository with a PEG base (PEG-R) formulated by a commercial compounding pharmacy[a] with a standard fusion protocol, then being placed in a mold and subsequently refrigerated; dissolved in water and administered rectally (H_2O-R); and as an oral capsule. Each administration was separated by a 7-day washout period and was given in a randomly assigned order, following a cross-over study design[9]. The dose administered was chosen to be consistent with the currently recommended dose for oral zonisamide administration in dogs.[6–8] Before each administration, a blood sample was drawn through a saphenous sampling catheter (time zero sample).

When administering a zonisamide suppository (PEG-R), the suppository was digitally placed within the rectum and the anus was held closed for approximately 5 minutes after dosing to minimize drug expulsion.[13] For H_2O-R, the contents of oral capsules corresponding to each dose were dissolved together in 10 mL of tap water. This solution was administered rectally in a 12 mL syringe, with a 16 inch (41 cm) 10 French Kendall Sovereign Feeding tube and Urethral Catheter, inserted approximately 10 cm into the rectum. The catheter was then flushed with 10 mL of tap water and the anus was again held closed for approximately 5 minutes to prevent early expulsion of the drug. For oral administration each dog had a zonisamide capsule placed in the back of the pharynx and was monitored for complete swallowing. All dogs were observed for adverse effects for a period of 24 hours after drug administration and daily throughout the 1-week washout period.

Thirteen blood samples were collected: at 3, 5, 10, 15, 30, 45, 60, 120, 240, 360, 480, 720, and 1,440 minutes after drug administration. All blood samples were centrifuged no more than 30 minutes after being drawn, and the plasma was harvested and frozen separately, at −20°C. Samples were shipped to the Clinical Pharmacology Laboratory at Auburn University.[b] Serum samples were thawed at room temperature and then vortexed to assure homogeneity. Zonisamide was analyzed by high-performance liquid chromatography (HPLC). The HPLC system was manufactured by Waters corporation and the separation was achieved with a C8, 5 μm, 250 × 4.6 mm ID reverse phase chromatographic column, protected by a precolumn C8, 5 μm, 4 × 2.0 mm operated at room temperature. Sample preparation was accomplished by using solid phase extraction (SPE) on a C18 SPE column. Samples were eluted from the columns by using methanol direct to the HPLC vial. The mobile phase was a mixture composed of 70% water and 30% acetonitrile. Flow rate was set at 1.0 mL/min. Drug was detected by using ultraviolet spectroscopy at 245 nm. The unknown concentrations in dog samples were quantitated by comparing the signal to standards prepared by the addition of known amounts of Zonisamide to canine plasma/plasma, to achieve known concentrations ranging from 0.4 to 180 μg/mL. The linear correlation coefficient was 0.9995. The limit of detection was 0.2 μg/mL. The lower and upper limit of quantitation were 0.4 and 180 μg/mL, respectively. The CV% for 0.4, 10, 50, 100, and 180 μg/mL were 13.09%, 5.13%, 2.62%, 3.06%, and 5.1%, respectively.

Plasma drug concentration versus time data was subjected to standard noncompartmental pharmacokinetic analysis by commercially available linear regression software.[c] Pharmacokinetic parameters of interest included maximum concentration (C_{max}), time to maximum concentration (T_{max}), disappearance half-life ($T_{1/2}$), area under the curve (AUC), and mean residence time (MRT). Other common pharmacokinetic parameters such as volume of distribution, clearance, and a true half-life could not be determined without intravenous drug administration. The mean bioavailability of the rectal formulations was determined relative to oral drug administration by comparing the area under the curve after each rectal dose to that after oral administration.

Statistical analyses for C_{max}, $T_{1/2}$, AUC, MRT, and relative bioavailability were conducted by a commercially available statisti-

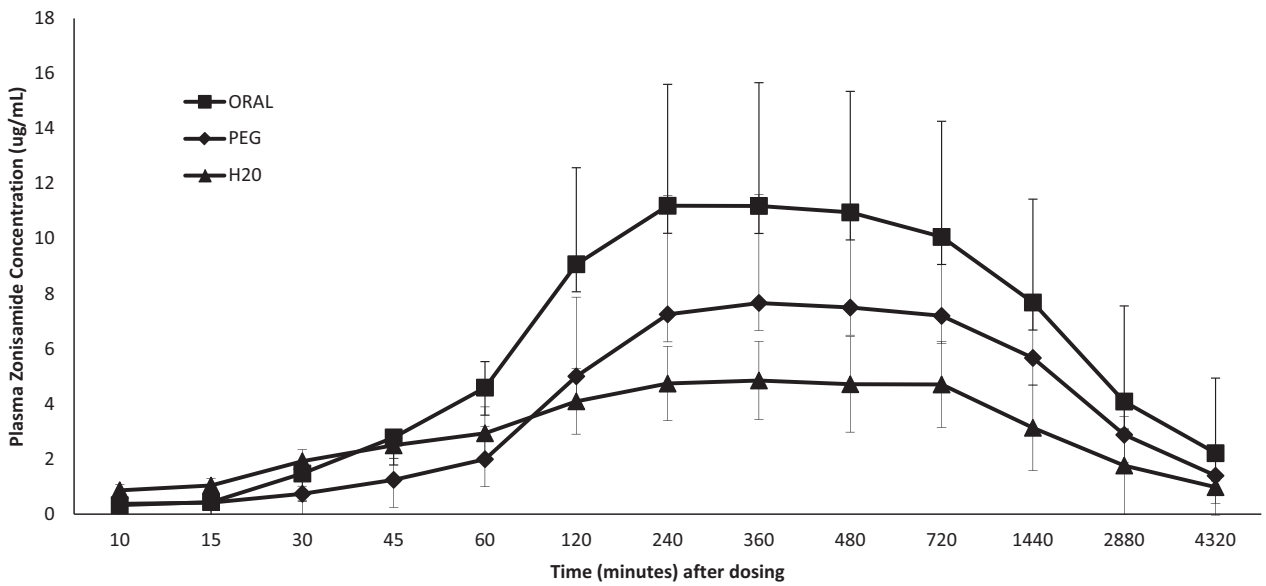

Fig 1. Mean plasma zonisamide concentrations for Oral, PEG-R, and H_2O-R routes.

Table 1. Summary of pharmacokinetic parameters.

Variable	Oral	PEG-R	H_2O-R
C_{max} (μg/mL)	11.56 ± 4.04[a]	8.13 ± 3.78	5.00 ± 1.83[a]
T_{max} (hours)	5.75 ± 2.92	8.5 ± 6.82	7.25 ± 3.01
$T_{1/2}$ (hours)	26.8 ± 4.7	24.3 ± 2.7	27.7 ± 6.3
AUC (min × μg/mL)	31,668 ± 12,051[a]	21,399 ± 8,402	13,689 ± 4,486[a]
MRT (minutes)	2,421.5 ± 406.7	2,255.9 ± 235.4	2,418.7 ± 491.3

[a]Significantly different, $P = .004$.

cal software program.[d] A Kolmogorov-Smirnov test was used to ensure the data were normally distributed. A repeated measures ANOVA was used to evaluate differences in pharmacokinetic variables between each route of administration. When differences were found, Bonferroni's multiple comparison tests was used to determine if these differences were significant. Because the T_{max} data were not continuous, a nonparametric Friedman's test[e] was used to identify if differences were significant. The relative bioavailability of rectal formulations was compared with a paired *t*-test.

Results

No adverse effects were noted with any route of zonisamide administration. The mean plasma drug concentrations attained with each formulation are plotted in Figure 1. The pharmacokinetic data for each formulation are summarized in Table 1. There were no significant differences in the T_{max}, $T_{1/2}$, and MRT among the 3 formulations. Significant differences in C_{max} were limited to oral compared to H_2O-R administration ($P = .0035$, 95% CI: 2.66–10.45). Similarly, AUC was significantly greater after oral administration compared to H_2O-R dosing ($P = .0037$, 95% CI: 7,200.64–28,757.19). The mean relative bioavailability of PEG-R (85 ± 69%) was higher than that of H_2O-R (53 ± 37%) ($P = .039$, 95% CI: 0.022–0.63).

Discussion

This study demonstrates that zonisamide is absorbed after rectal administration to dogs, without apparent adverse effects.

In light of the higher mean C_{max} and AUC (an indicator of bioavailability) obtained with oral administration in comparison to H_2O-R administration, absorption of this drug appears to be limited when it is given rectally dissolved in water. PEG, in contrast, might be a better vehicle for rectal zonisamide administration in dogs, as it had a higher relative bioavailability than after H_2O-R dosing and its C_{max} did not differ significantly from that obtained after oral dosing. The superior performance of the PEG-R formulation over the H_2O-R formulation is not surprising given the known cosolvency property of PEG[13].

In this study, the mean T_{max} was not reached for over 7 hours with both rectal formulations. PEG was chosen as a drug vehicle in this study because of rapid and effective drug absorption with PEG after rectal administration of zonisamide in rats.[13] Water was also evaluated as a vehicle because of its accessibility in clinical practice in comparison to specially formulated suppositories. However, in comparison to reports of zonisamide administered rectally in rats, where there was bioavailability of approximately 100%, we observed a lower bioavailability with either PEG or water formulations in dogs.[13] Possible reasons for this include inherent differences in the transmucosal absorptive capacity between the gastrointestinal systems of the dogs, the volume of feces in the rectum of the dogs at the time of administration, or the possibility of zonisamide inadvertently being expelled after rectal administration. All of the prior are natural limitations of administering medications rectally. In addition, it is possible that the PEG formulation used in our study could have been improperly manufactured or that improper handling after preparation could have led to premature degradation of the suppository. Unfortunately, additional suppository samples were not available for determining the accuracy of drug concentration after the completion of this study.

The delay in rectal absorption seen in this study is comparable to that seen with oral administration, but it must be considered if rectal administration of zonisamide is to be performed in a clinical situation, as it should not be used as a sole treatment for status epilepticus. Rather, this route of administration could provide a means of beginning treatment with a long acting antiepileptic drug in dogs that are unable to receive medications PO. In addition, the half-life of zonisamide after rectal administration was similar to that obtained with oral administration. Based on the mean disappearance half-life obtained in this study (24.3 and 27.7 hours for PEG-R and H_2O-R, respectively), twice-daily dosing should be sufficient for maintaining therapeutic concentrations by rectal administration of this drug.

Ideally, bioavailability is best determined by comparing AUC measurements obtained after intravenous and nonintravenous administration of a drug.[14] Unfortunately, as zonisamide was not available for intravenous administration at the time this study was performed, we were limited to calculating a relative bioavailability by comparing our rectal formulations to oral administration. Although more precise pharmacokinetic data could have been obtained after intravenous administration, including an estimate of first-pass metabolism, this study provides useful insight as to the pharmacokinetic profile of rectally administered zonisamide.

In summary, a single rectally administered dose of zonisamide is well tolerated and does not result in any noticeable adverse effects in this small group of healthy

dogs. Given that rectally administered zonisamide dissolved with water has a lower bioavailability compared to oral administration, a higher dose is likely necessary to reach therapeutic levels. Twice-daily dosing appears to be appropriate for this route of zonisamide administration to dogs. This study only evaluated single-dose pharmacokinetics. Given the delay to maximum concentration (T_{max}) seen with all methods of delivery, the administration of zonisamide would not be a suitable immediate treatment for status epilepticus. Additional studies would be needed to describe the pharmacokinetics of repeated dosing of any method of administration.

Footnotes

[a] The Compounding Shop, 4518 N. Henry Blvd, Suite 106, Stockbridge, GA 30281
[b] Clinical Pharmacology Laboratory, 109 Greene Hall, Auburn University, AL 36849–5518
[c] WinNonLin, Pharsight Corporation, Mountain View, CA
[d] MedCalc Software, Mariakerke, Belgium
[e] Mathlab R2008b, The MathWorks, Natick, MA

Acknowledgments

The study was funded with internal funds provided by the small animal neurology service. No external grant money was used.

Conflict of Interest Declaration: The authors disclose no conflict of interest.

Off-label Antimicrobial Declaration: The authors declare no off-label use of antimicrobials.

References

1. Thomas WB, Dewey CW. Seizures and narcolepsy. In: Dewey CW, ed. A Practical Guide to Canine and Feline Neurology, 2nd ed. Ames, IA: Wiley-Blackwell; 2008:237.

2. Jaggy A, Bernardini M. Idiopathic epilepsy in 125 dogs: A long-term study. Clinical and electroencephalographic findings. J Small Anim Pract 1998;39:23–29.

3. Mariani C. Terminology and classification of seizures and epilepsy in veterinary patients. Top Companion Anim Med 2013;28:34–41.

4. Dewey CW. Anticonvulsant therapy in dogs and cats. Vet Clin North Am Small Anim Pract 2006;36:1107–1127, vii.

5. Podell M, Fenner WR. Bromide therapy in refractory canine idiopathic epilepsy. J Vet Intern Med 1993;7:318–327.

6. Dewey CW, Barone G, Smith K, Kortz GD. Alternative anticonvulsant drugs for dogs with seizure disorders. Vet Medicine 2004;99:786–793.

7. 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.

8. Walker RM, DiFonzo CJ, Barsoum NJ, et al. Chronic toxicity of the anticonvulsant zonisamide in beagle dogs. Fundam Appl Toxicol 1988;11:333–342.

9. Biton V. Clinical pharmacology and mechanism of action of zonisamide. Clin Neuropharmacol 2007;30:230–240.

10. Hwang H, Kim KJ. New antiepileptic drugs in pediatric epilepsy. Brain Dev 2008;30:549–555.

11. Shorvon D, Perucca E, Engel J Jr. The Treatment of Epilepsy, 3rd ed. Ames, IA: Wiley-Blackwell; 2009:1075.

12. Mori A, Noda Y, Packer L. The anticonvulsant zonisamide scavenges free radicals. Epilepsy Res 1998;30:153–158.

13. 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.

14. Boothe DM. Small Animal Clinical Pharmacology and Therapeutics. Philadelphia, PA: W.B. Saunders Company; 2001:806.