

**CLINICAL PHARMACOKINETICS AND SAFETY OF ZONISAMIDE IN
APPARENTLY NORMAL DOGS FOLLOWING SINGLE AND MULTIPLE DOSING**

A Thesis

by

JEREMY DANE PERKINS

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

August 2004

Major Subject: Veterinary Physiology

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Approved as to style and content by:

Dawn Boothe
(Co-Chair of Committee)

Deborah Kochevar
(Co-Chair of Committee)

Jeffrey Musser
(Member)

Glen Laine
(Head of Department)

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Major Subject: Veterinary Physiology

ABSTRACT

Clinical Pharmacokinetics and Safety of Zonisamide in Apparently Normal Dogs Following
Single and Multiple Dosing. (August 2004)

Jeremy Dane Perkins, B.S., Texas A&M University

Co-Chairs of Advisory Committee: Dr. Dawn Boothe
Dr. Deborah Kochevar

The purpose of this study was to design a dosing regimen and evaluate the safety of zonisamide (ZNS) following multiple dosing and to determine appropriate monitoring methods. Clinical pharmacokinetics were studied in 8 adult dogs (4 male and 4 female) ranging from 3 to 4 years of age using a randomized crossover design following single intravenous (IV) and oral administration, 6.85 and 10.25 mg/kg, respectively. Samples were collected intermittently for 48 hours. Dogs were then dosed orally (10.17 mg/kg) twice daily for 8 weeks. Blood samples were collected weekly and at discontinuation of the drug. Additionally, urine was collected to determine 24 hour urine ZNS clearance following IV administration. Safety was based on clinical pathology, thyroid and urine testing during both studies.

ZNS was measured using high performance liquid chromatography in serum, plasma, erythrocytes (RBC) and whole blood. Data were subjected to standard non-compartmental pharmacokinetic analysis using computer assisted linear regression (WinNonLin[®]). Comparisons were made in different compartments using one-way ANOVA to identify any differences. Safety parameters at study beginning and end were compared using a Student t-test.

ZNS concentrations differed among blood compartments after single dosing, with oral maximum concentration (C_{max}) being greatest in RBC (28.73 μ g/ml) and least (14.36 μ g/ml) in plasma. Volume of distribution also differed, being greater (1096.05ml/kg) in plasma and least

in (379.23ml/kg) RBC. Clearance of ZNS was 57.55ml/hr/kg from plasma and 5.06ml/hr/kg from RBC. Elimination half- life in plasma was 16.4 hr in serum and 57.4 hr in RBC. Bioavailability was 126.8% for RBC and 189.6% for plasma.

Following multiple dosing, at steady-state, C_{max} averaged 65.8 μ g/ml with fluctuations of 17.2% between dosings. Accumulation of ZNS was 3.5 (plasma) and 4.3 (RBC). Concentrations did not differ among blood compartments at the end of multiple dosing. Although differences did occur across time in clinical pathology tests, all were within normal limits at study end except for T4.

In conclusion, ZNS dosed at 10 mg/kg twice daily for dogs would maintain therapeutic levels (10 to 70 μ g/ml) recommended in human epileptic patients. Therapeutic monitoring would be best measured in serum or plasma accompanied with thyroid and urine testing.

I dedicate this to my wife, Dana,
for all the support and happiness
she brought me in pursuing my dreams.

ACKNOWLEDGEMENTS

I would like to thank the Morris Animal Foundation for a grant in aid for this study. I would like to thank the professors and staff who provided help and guidance, including, Dawn Boothe, Maya Scott, Tiffany Finch, Crisanta Cruz and Ilona Pertrikovics.

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CHAPTER I

INTRODUCTION

Mechanism of Action

Developed in Japan, zonisamide (ZNS), 3-sulfamoylmethyl- 1, 2 benzisoxazole, is a sulfonamide-based anticonvulsant.¹ Dainippon Pharmaceutical Co. synthesized the drug in 1974. The Food and Drug Administration approved ZNS in 1998 for treatment of seizures related to human epilepsy.¹ The efficacy of ZNS is similar in the treatment of human epilepsy to phenobarbital and better than other classic drugs including valproic acid and phenytoin.¹ Zonisamide appears to inhibit neuronal voltage-dependent sodium and T-type calcium channels.² Additionally, ZNS modulates the dopaminergic system³ and accelerates the release of γ -amino butyric acid (GABA) from the hippocampus.^{3,4} A potential advantage of ZNS is free radical scavenging which protects against the destructive nature of radicals, especially in neuronal membranes.⁵ Finally, ZNS blocks the propagation of seizures from cortex to subcortical areas of the brain.⁶ Although its mechanism of action is not certain, its antiepileptic efficacy, when combined with other anticonvulsants, supports an action that is similar to phenytoin⁷ or valproic acid.⁸ As such, an advantage to ZNS is that it is less likely to affect normal neuronal activity.⁷

Clinical Pharmacology

The clinical pharmacology of ZNS has been investigated in humans and laboratory animals(mice, rats and primates) with limited data reported in dogs.⁹⁻¹² In humans, oral absorption is rapid and complete and is minimally impaired by food.¹ Maximum concentrations occur between 2.8 to 3.9 hours.¹ Data regarding oral bioavailability is limited but was 84% in

This thesis follows the style and format of the Journal of Veterinary Internal Medicine.

humans in one report.⁸ The volume of distribution (Vd) approximates 1.47 L/Kg, indicating distribution to total body water.⁸ After 12 hours, the concentration of ZNS in the brain, liver, adrenal gland was twice that in plasma, suggesting good distribution to these organs.¹⁰ In addition, CNS penetration by transcapillary action across the blood brain barrier quickly equilibrates.¹³ Rat studies indicate little efflux of ZNS from the brain, implying the drug is bound, or through other means, retained in the brain.¹³

Protein binding is $47.9 \pm 0.9\%$ in rats and between 40 and 60% in humans¹⁴, suggesting that protein binding will not limit the rapid movement into the brain. Further, when combined with other highly protein-bound drugs such as phenobarbital, unbound ZNS did not change.¹⁰ The disposition of ZNS is complicated by binding to erythrocytes (RBC) as well as plasma proteins. As in the brain, ZNS concentration remains greater in RBC despite low serum concentrations.¹¹ In a study comparing mean whole blood and plasma or serum concentrations using area under the curve (AUC), RBC concentrations in whole blood were twice as high as plasma¹⁵ and serum.¹¹ This binding has been shown to have two components: saturable and non-saturable.¹³ Because of the large variation between serum and whole blood the saturable portion may reflect ZNS binding to carbonic anhydrase in epileptic patients.¹¹ The accumulation of ZNS in RBC complicates calculation of volume of disposition (Vd). For example, Vd in humans declined apparently due to non-linear accumulation in RBC.^{7,8} The decrease in VD appears to lead to increased plasma drug concentrations.⁷ Accumulation in RBC, however, does not appear to impair distribution into the brain: approximately 50% of drug reaching the CNS in rats originated from RBC during a single transcapillary passage after determination concentrations in brain tissue.¹³ The accumulation of drug in RBC is reversible, and the complex relationship between ZNS and RBC may make therapeutic drug monitoring of plasma or serum advantageous.^{8,16}

Metabolism of ZNS includes both phase I and phase II hepatic metabolism with cytochrome P450 3A4 being the major isozyme¹⁷ and a glucuronidated compound the major metabolite.¹⁸ Renal elimination and recovery of ZNS indicates parent drug recovery of 35%.¹ Using radio labeled (carbon) ZNS administered to dogs, 83% of the drug was excreted in 72 hour urine as either the parent compound or metabolites. The remaining proportion was recovered in feces.¹⁰

The terminal half-life of ZNS in the dog following a single oral dose (20mg/kg) administration differed depending on the tissue studied with the shortest being 15 hours for plasma^{10,12} and the longest, 42 hours for RBC.¹⁰ The longer elimination half-life for plasma and RBC is beneficial because it allows a convenient dosing interval while minimizing dramatic fluctuations in ZNS concentrations that might cause recurrence of seizures. Twice daily dosing resulted in a 14% fluctuation of plasma drug concentrations in humans versus 27% with once daily dosing.⁸ Twice daily dosing is preferred in humans.¹⁹ Early studies suggesting that therapeutic concentrations for ZNS would be 10-70 $\mu\text{g/ml}$ ²⁰ appear to have been confirmed with most recent studies indicating a therapeutic range from 16.5 to 49.6 $\mu\text{g/ml}$ ⁹ when dosed twice daily.

Non-linear pharmacokinetics have been reported in some human patients, particularly with chronic dosing, resulting in disproportionate, and thus unexpected, increases in drug concentrations compared to changes in dose.⁷ Although the pharmacokinetics of ZNS apparently have not been reported in dogs, plasma concentrations, when receiving 75 mg/kg, never reached steady state over the course of thirteen weeks, while dosages of 10 to 30 mg/kg attained steady state by week 13 and were proportionate to dose.¹² The 75mg/kg dose concentrations were disproportionate to the concentrations of 10 and 30 mg/kg with the other dosages and may account for the non-linear pharmacokinetics seen in the previous article.

Safety

In a toxicity study in the dog, doses of 10 to 30 mg/kg did not appear to cause serious side effects.¹² However, at 75 mg/kg dosed twice daily, hypoalbuminemia and slightly higher alkaline phosphatase activity indicated possible hepatic involvement.¹² Yet, the therapeutic index appears wide in dogs⁷ with the lethal dose in dogs to be over 1000 mg/kg.²¹ Chronic toxicity studies in dogs receiving up to 100 mg/kg for eight weeks revealed transient sporadic emesis and increased liver weight.²¹ In contrast, chronic dosing at 75 mg/kg once daily for 52 weeks in beagles resulted in decreased appetite and weight loss, which resolved when the total daily dose was divided quarterly.

Following approval of ZNS in humans, post-market studies have indicated rare adverse effects in selected organ systems. Renal tubular acidosis has been reported in the occasional patient, with resolution occurring once the drug is discontinued.²² Additionally, a series of cases of urolithiasis (calcium phosphate or calcium oxalate) has been described in children receiving ZNS for refractory epilepsy.²³ This may reflect the carbonic anhydrase inhibitory activity of ZNS, by increasing calcium excretion with a subsequent decrease in urine citrate in the production of uroliths.²⁴ In another study, renal calculi occurred in 3% of human patients receiving the drug⁷, although most uroliths were only a few millimeters in size. As with renal tubular acidosis, uroliths and associated clinical signs resolved with discontinuation of ZNS therapy.²⁵ In 3 out of 10 dogs treated with 75 mg/kg of ZNS, the urinary bladder was grossly affected and confirmed microscopically for damage; however, the author considered the changes to be insignificant in this study.¹²

Historically, sulfonamide-based drugs have been associated with reductions in circulating levels of thyroid hormones (T4 and T3) in dogs and humans.²⁶ This reduction of the hormone is possibly due to the impairment of thyroglobulin iodination and coupling of tyrosines

in the thyroid tissue.^{27,28}

ZNS appears to be minimally involved in drug interactions which are typical of several other anticonvulsants (phenytoin, valproic acid) with high protein affinity.¹⁰ Studies in vitro have demonstrated that ZNS binding to protein was unaffected by phenytoin and valproic acid concentrations.¹⁰ Interactions due ZNS concentration in RBC have caused anticonvulsant concentrations in serum to slightly increase.²⁹ ZNS does not appear to affect its own metabolism nor the metabolism of other drugs in animals or humans.³⁰ However, the enzyme-inducing anticonvulsant phenobarbital, shortened the half-life from 27 to 36 hours in humans¹⁴ and lowered plasma concentrations of the drug.³¹

Justification

Antiepileptic failure can be life threatening. A common cause of therapeutic failure is a decline in antiepileptic drug concentrations below the minimum therapeutic range. The disposition of anticonvulsants can be complex, particularly with chronic dosing. Further, because antiepileptic drugs are given long term, the risk of adverse reactions and drug interactions increases. The most effective use of an antiepileptic is based on scientific studies that determine the proper dose and interval necessary to achieve and maintain concentrations throughout the dosing interval in plasma and central nervous system (CNS). The drug concentrations should be above the therapeutic minimum but below the therapeutic maximum. Although some studies of ZNS have been performed in dogs, these studies were largely implemented as part of the approval process for the drug in humans, thus are limited to toxicities and associated side effects. Currently, the amount of pharmacokinetic information available in the dog is insufficient to justify evidence-based administration of ZNS for patients suffering from idiopathic epilepsy.

Hypothesis

ZNS will safely achieve and maintain in canine plasma throughout the dosing interval concentrations considered therapeutic in humans when dosed orally at 10 mg/kg every 12 hours.

Objectives

1. To determine the oral dose and interval of ZNS necessary to maintain antiepileptic therapeutic concentrations, with minimal fluctuation during a dosing interval. Specific objectives are to:
 - a. to determine the oral bioavailability;
 - b. determine a dose necessary to achieve a C_{\max} within the therapeutic range described in humans;
 - c. determine the maximum or a convenient oral dosing interval that will minimize fluctuation in drug concentrations throughout the interval;
 - d. determine the major route of elimination in order to predict potential drug interactions or adverse reactions in unhealthy patients;
 - e. based on a-c, recommend an oral dosing regimen;
 - f. confirm the appropriateness of the oral dosing regimen when administered for several weeks;
2. To determine the safety of ZNS when administered following multiple doses; Specific objectives are to:
 - a. describe changes in clinical laboratory tests following multiple dosing that might be indicative of undesirable side effects;
 - b. describe the effect of multiple ZNS dosing on thyroid status;
 - c. determine the effect of ZNS on urinary excretion of calcium and citrate;
and
3. To determine the most appropriate method of monitoring ZNS when used as an anticonvulsant. Specific objectives are to:
 - a. compare the disposition of ZNS as measured in all blood compartments (plasma, serum, red blood cells and whole blood); and
 - b. to determine the most appropriate (peak versus trough) sampling times.

The combination of the data collected will provide information in the development and implementation of a dosing regimen to medicate dogs suffering from idiopathic epilepsy.

CHAPTER II

PREPARATORY STUDIES

Introduction

The complex chemistry and kinetics of ZNS required several preliminary studies prior to implementation of the clinical pharmacokinetic studies. These included validation of the assay in multiple canine blood compartments (plasma, serum, whole blood and RBC) and urine. Further, because ZNS is not commercially available as a solution intended for IV use, and pure powder is not commercially available in amounts reasonably affordable, an IV preparation was prepared from oral tablets. Finally, the fraction of unbound ZNS was determined in canine plasma.

Materials and Methods

Chemicals and reagents

A commercial preparation of zonisamide (Zonegran[®], Dainippon Pharmaceutical, Osaka, Japan) distributed by Elan Corporation (Dublin, Ireland) was used for the clinical pharmacokinetic and safety studies. Sodium zonisamide was purchased from Sigma-Aldrich (St. Louis, Missouri) for use as a standard in analytical measurement of biologic fluids. High performance liquid chromatographic (HPLC) analytical grade methanol and acetonitrile were purchased from E. Merck (Darmstadt, Germany). Citric acid monohydrate (E. Merck), potassium dihydrogen phosphate (E. Merck), and tetrabutylammonium hydrogen sulfate (Sigma –Aldrich), 5- Sulfosalicylic acid (Sigma- Aldich) were all of analytical grade.

Analytical equipment

The proper wavelength for detection of ZNS was determined by dissolving the sodium ZNS in methanol and subjecting it to dynamic spectrophotometric analysis using a Beckman DU

70 Spectrophotometer (Fullerton, California). The optimal signal was recorded at a wavelength of 245 nm.

Reverse phase HPLC was used to quantify the concentration of ZNS in unknown samples. The HPLC system from Waters (Milford, Massachusetts) consisted of a pump (600 controller), an autosampler (717 plus Autosampler) and an ultraviolet detector (Dual λ Absorbance detector). The ultraviolet detector was set at 245 nm with a sensitivity of 2.00AUFS an analytical column μ -Bondapak™ C₁₈ (3.9 X 300mm, 125 Å 10 μ m particle size) with a Sentry™ Guard column, 3.9x 20mm (μ Bondapak™ C₁₈ ,125 Å 10 μ m particle size). The mobile phase consisted of purified distilled water and methanol (70:30, v/v) filtered through a 47 mm, 0.45 μ m GH Polypro Hydrophilic Polypropylene Membrane filters from Pall Life Sciences (Ann Arbor, Michigan). The mobile phase was degassed with a Waters inline degasser. The flow rate was set to 1.4 ml/min.³²

Preparation of standards

Canine blank plasma, whole blood, serum and urine were provided through the Texas A&M College of Veterinary Medicine Clinical Pharmacology Laboratory and represented pooled samples collected from apparently clinically normal dogs. Initial development of an assay to quantify concentrations of drug in whole blood, plasma, serum and urine was done in several steps. A stock solution of 100 μ g/ml was prepared in methanol. The stock solution was diluted with methanol to obtain standard and control concentrations of 0.1, 0.3, 1, 3, 5, 10, 25, 30, 50, 70, 75, and 100 μ g/ml. A 60 μ l of each standard and control was analyzed by HPLC using the equipment and mobile phase listed above. In methanol, the relationship between ZNS and the signal was characterized by an $R^2 = 0.9999$ with a linear regression equation of $y = 33641x - 12716$. The percent recovery of ZNS in plasma compared to the methanol stock was 88.6 %. The stock solution was stored at -20 °C until needed for use in making standards and controls for

whole blood, serum, plasma, and urine.

Biologic standards

Blood and urine were collected via jugular venopuncture and urinary catheter, respectively. Whole blood was collected in lithium heparin Monoject® (Sherwood Medical, St.Louis, Missouri) tubes and clot tubes Monoject® (Sherwood Medical, St.Louis, Missouri). Standards and controls for whole blood were prepared from heparinized whole blood immediately after collection and prior to freezing to ensure that erythrocytes were not lysed in the process of freezing and thawing.³³ Plasma was removed from heparinized whole blood following centrifugation (Beckman GPR Centrifuge, Fullerton, California) at 1600g for 15 minutes. Plasma was harvested and stored frozen at -20 °C until needed. Serum was removed from a whole blood in a clot tube and centrifuged at 1600g for 15 minutes. Serum was harvested and stored frozen at -20 °C until needed. Urine was collected and pooled from three dogs and frozen at -20 °C until needed. Standards and controls of whole blood, plasma, serum, and urine were made using the stock solution of sodium ZNS. A set of standards and controls were obtained in concentrations of 1 - 100 µg/ml. Whole blood controls were 1, 3, 7, 30, 45, 70, and 90 (µg/ml). Plasma controls were 3, 7, 15, 20, 25, 45, 70, 75, and 90 (µg/ml). Serum controls were 2, 5, 7, 15, 18, 20, 25, 30, and 60 (µg/ml). Urine controls were 15, 45, 70, and 90 (µg/ml). The methanol from the pipetted standards and controls was evaporated using an N- Evap Analytical Evaporator® (South Berlin, Massachusetts) under a stream of nitrogen gas with vials containing standards immersed in a water bath maintained at 60 °C. Once dry, 1 ml of fresh whole blood and thawed plasma, serum, and urine were added to their respective test tubes. All samples were lightly vortexed for 2 minutes, and then allowed to equilibrate throughout the sample at room temperature for 30 minutes before being frozen at -20 °C until analyzed.

Serum, plasma and urine preparation

Standards and controls were allowed to thaw to room temperature before beginning the analytical preparation. Solid phase extraction with a Phenomenex® (Torrence, California) Strata C₁₈-E (55 µm, 70 Å) cartridge was conditioned with 1 ml of methanol then 1 ml of purified distilled water.³² An aliquot of 200 µl of serum, plasma, or urine sample was added to the cartridge followed by a 700 µl wash of purified distilled water. The sample was eluted from the cartridge under vacuum with 200 µl of methanol. A 60 µl sample of the elute was injected into the HPLC sample using the same equipment and method mentioned above.

Whole blood preparation

Whole blood samples, standards and controls were allowed to reach room temperature before analysis. Each then was vortexed for 30 seconds to homogenize the blood. A 300 µl aliquot of whole blood and 600 µl of an acetonitrile: methanol mixture (90:10)³⁴ was vortexed for 30 seconds then centrifuged using Beckman GPR Centrifuge (Fullerton, California) at 3000 RPM for 10 minutes at 5 °C. The supernatant was then added to a Phenomenex® (Torrence, California) Strata C₁₈-E (55 µm, 70 Å) cartridge which had been preconditioned with 2 mls of methanol. The sample was eluted with 1 ml of methanol into individual test tubes. The elute was evaporated using a N- Evap Analytical Evaporator (South Berlin, Massachusetts) under a stream of nitrogen gas with a water bath set to 60 °C until dry. The residue was then dissolved with 200 µl of methanol. A 60-µl injection of the sample was injected into the HPLC machine for analysis.

RBC concentration

The RBC concentrations were determined mathematical using plasma and whole blood

concentrations and each dog's hematocrit. The RBC equation is:

$$\text{RBC} = \frac{1}{H} [C_B - (1-H)C_p]$$

RBC = concentration in packed red blood cells, H= fractional hematocrit, C_B = whole blood concentration of ZNS, and C_p = plasma concentration of ZNS (see Appendix B).

Intravenous solution

Ten capsules of Zonegran[®] (ZNS) 100mg were emptied into a beaker. Methanol (30 ml) was added to the beaker and stirred vigorously with a stir bar for 10 minutes. The supernatant was removed and filtered through a Pall Gelman Laboratory (Ann Arbor, Michigan) 13mm GHP Acrodisc[®] 0.45 μ m syringe filter. The supernatant was then evaporated to dryness under a stream of nitrogen. Once dry, methanol was added to the residue and vortexed for 10 minutes and then allowed to rest for 20 minutes. The supernatant was removed and filtered with a 0.45 μ m syringe filter. The filtrate was dried under a stream of nitrogen. The residue was removed from the holder and weighed. The residue was treated as pure ZNS.

To assure all drug was dissolved (thus avoiding IV injection of particulate matter), several solvents were investigated for their potential safe use. These included dimethyl sulfoxide (DMSO), ethanol and saline. After adding an equal amount of ZNS to three test tubes, 20 mls of DMSO, ethanol, and saline were added. The tubes were vortexed for 2 minutes and then allowed to rest for 30 minutes at room temperature. The solubility of zonisamide in ethanol and DMSO were seen to be acceptable based on absence of visible precipitates. Impact of each of these solvents on RBC integrity was then considered in order to avoid solvent-induced release of ZNS that had accumulated in RBC. Because DMSO causes RBC hemolysis when administered as more than a 10% solution,³⁵ ethanol was considered to be the more likely candidate.³⁶ Despite its clinical use for treatment of ethylene glycol toxicity (20 % ethanol) in dogs (5.5 ml/kg every 4

hours or 1.1 gm/kg every 4 hours)³⁷, ethanol is associated with adverse events and is characterized by a LD100 of 5.365 g/kg.³⁸ Based on the ethylene glycol dose, no more than 4 g/kg of ethanol was determined to be safe for the test subjects involved (see Appendix A).

A targeted solution of 8 mg/ml of ZNS in ethanol was prepared as follows. The measured amount ZNS was dissolved with 33% ethanol⁹ until no visible drug was present. Saline was then added at a ratio of 2:1 (saline: ethanol) to dilute the effects of ethanol and to lessen hemolysis. The ZNS solution was filtered through a Whatman[®] (Clifton, New Jersey) 25mm 0.2µm PES syringe filter. Afterwards, the solution was stored at 5° C until analysis or administration.

To confirm both the concentration of drug and purity of the drug, standards and controls were made using the commercially available ZNS dissolved in the ethanol (1): saline (2) solution used for the IV preparation. A set of standards and controls were made from 1- 150 µg/ml. Controls were 3, 15, 45, 85, and 125 (µg/ml). Several aliquots of the intravenous solution were added to verify the concentration of the intravenous solution in duplicate. Based the on the regression line equation for the sum of least squares ($y = 91546x - 96640$, $R^2 = 0.9985$) for the standards, the IV solution concentration was extrapolated to 3.9 mg/ml, which was confirmed following formulation of a second intravenous solution with a concentration of 4.0 mg/ml.

Total protein binding

Total protein binding in canine plasma was based on equilibrium dialysis^{39,40} using a 96 well-plate dialyzer (Harvard Apparatus, Holliston, Ma) with molecular cutoff of 5000 Daltons. To determine the percentage of unbound drug, six concentrations within the therapeutic range⁹ were chosen to be evaluated. Aliquots of the stock solution ranging from 10– 50 µg /ml were dried under a stream of nitrogen. Phosphate buffered saline (PBS), 0.1M, was added to the

residue until dissolution. Standards and controls were made in the same manner as above. Frozen plasma was removed and thawed at room temperature.

A 200 μ l aliquot of PBS- ZNS solutions was added to one side of the 96- well-plate and capped. A 200 μ l aliquot of drug-free plasma was added to the other side and capped. The plate was then allowed to equilibrate for 24 hours at 37 °C. After 24 hours the samples were removed and prepared for analysis by HPLC. The preparation and equipment were the same techniques used for serum, plasma, and urine previously discussed.

Results

Validation of analytical assay

Analysis of ZNS in whole blood, plasma, and serum were validated using HPLC. Standard and control concentrations were compared to known concentrations using an equation derived from the sum of least squares. Controls and the accompanying standard curve were considered acceptable if the percentage error (difference between actual and predicted divided by the actual) was less than $\pm 15\%$. Intra- assay and inter-assay variability were determined.

Intra-assay accuracy for serum controls predicted within $95.5 \pm 4.8\%$ (range 91.3 – 106.0%) with a regression equation $y = 44114x + 19636$. Plasma was $97.7 \pm 6.0\%$ (range 88.0- 111.0%) with a regression equation of $y = 43175x + 8707.9$. Whole blood was $91.4 \pm 6.8\%$ (range 83.4 - 100.7%) with a regression equation $y = 64586x - 16687$.

Inter-assay controls precision for serum was $99.5 \pm 3.7\%$ (range 94.5 – 105.8 %). Plasma was $99.5 \pm 5.6\%$ (range 92.2 – 109.8). Whole blood precision was $95.2 \pm 6.4\%$ (range 84.2 – 101.5%). Urine results was $103.2 \pm 8.86\%$ (range 95.05- 113.05%) with a regression equation $y = 81270x - 630244$, refer to appendix C for all data. The retention time of ZNS was 7.8 minutes regardless of the body fluid (Fig 1).

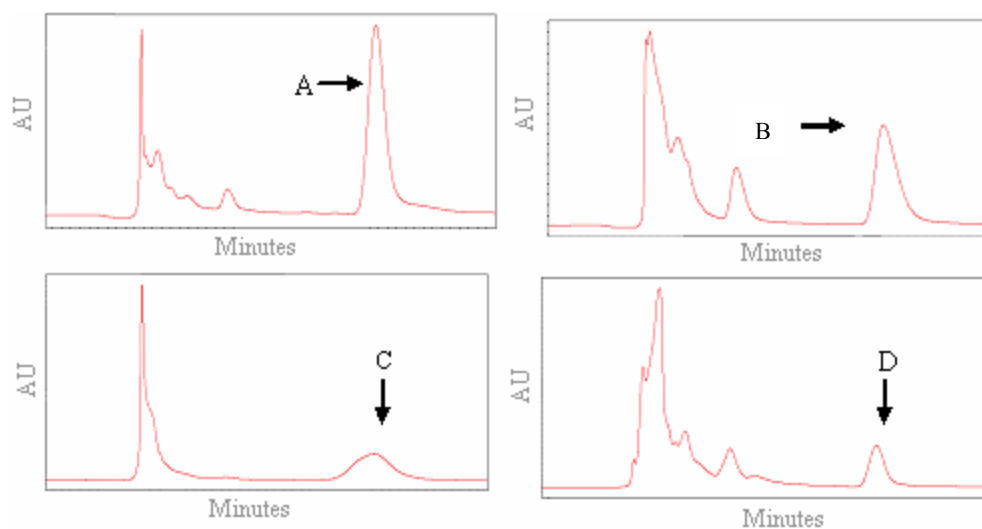


Fig 1. Serum, plasma, whole blood, and urine chromatographs. Each chart represents an example of four different samples (A=serum ZNS, B= plasma, C= whole blood, D= urine) tested through HPLC analysis. The retention time is 7.8 minutes for the ZNS samples.

Protein binding

Using an established therapeutic window for humans, the percentage of unbound versus bound zonisamide was determined. With concentrations ranging from 6 to 55 $\mu\text{g/ml}$, the average unbound portion of zonisamide available for pharmacological activity was $60.48 \pm 13.47\%$ (Table 1).

Intravenous preparation results

Both of the tested IV solutions were compared against what was weighed and calculated and were found to be 15% different than what was initially calculated. The final concentrations of the prepared solution were, 3.9 and 4.0 mg/ml.

Table 1. Protein binding of ZNS. ^a

Therapeutic window concentration ($\mu\text{g/ml}$)	Unbound Drug %	Protein Bound Drug %
6	58.811	41.189
10	76.093	23.907
15	43.466	56.534
25	55.515	44.485
35	73.104	26.896
40	47.886	52.114
50	77.808	22.192
55	51.164	48.836
Average	60.481	39.519
Standard Deviation	13.447	13.447

^aProtein binding for the therapeutic window of 6-55 $\mu\text{g/ml}$ concentration in the body. Bound and unbound percentages are listed for individual concentrations.

Discussion

Validation of assay

Establishing assays for the prediction of concentrations in plasma, serum, urine, and whole blood are important for the modeling of the disposition of ZNS in the dog. All the tested assays were capable of controls predicting within $\pm 15\%$ of the expected concentration. The percentage difference was acceptable and was used in determining the ZNS concentrations for the appropriate blood compartment.

Protein binding

Binding of ZNS to plasma proteins was determined to estimate the amount of drug that would be free for pharmacological activity as well as to predict any potential drug interactions at the level of protein binding.^{39,41} Studies revealed the unbound fraction of drug to be $60.48 \pm$

13.4%. The percentage of the drug bound is close to the percentage bound by rats. In rat studies, protein binding was found to be $47.9 \pm 0.9\%$.¹⁰ The proportion of protein binding was not deemed sufficient to contribute to drug interactions, but the apparent volume of distribution and clearance could be lowered because of the proportion bound hindering movement from circulation to specific active sites.

Intravenous preparation

In developing the intravenous solution, care was taken to ensure safety for the dogs. Filtering of the finished preparation was performed to ensure that any microscopic particulate was eliminated from the solution. However, this does not guarantee removal of pyrogens. Analysis of the final finished solution indicated that the final concentration of 4 mg/ml was lower than that anticipated based on preparation (8 mg/ml). Reasons for the differences might include a calculation error (less likely as FDA approved capsules were used as measured units and duplication of the procedure yielded strikingly similar concentrations). It also is possible that apparent soluble components in the oral form were soluble in methanol and were incorporated in to the final weight of the powder used in the preparation of the IV solution. Because purity was not established, this is possible, but the magnitude of contamination was sufficiently large that HPLC analysis should have indicated some type of competing material. However, no peaks other than ZNS were evident. The most likely explanation of lower than expected concentrations of ZNS following extraction from oral capsules was decreased recovery (50%) due to binding of active to inactive ingredients. However, less than ideal recovery was not a detractor to the success of the study; the IV dose was based on a concentration of 4 mg/ml of ZNS in ethanol. At this dose, each animal would also receive 5 mg/kg of ethanol as a 33 % solution.

CHAPTER III

SINGLE DOSE PHARMACOKINETIC DATA

Introduction

Single dose evaluations are used to characterize pharmacokinetic data to develop a dosing regimen for long-term therapy. Data collected was used to calculate absolute bioavailability for ZNS to determine the extent of absorption following oral administration. Further, the single dose study provided a basis for a dosing regimen (dose and interval) when ZNS is used as an antiepileptic drug. The design of the study allowed for non-biased and independent evaluation of data collected

Materials and Methods

Research subjects

All research protocols were approved by the Texas A&M University Lab Animal Care Committee. Eight (4 male and 4 female) hound dogs (29.41 ± 5.36 kg) ranging from 3 to 4 years of age were studied using a randomized cross-over design. Dogs were apparently healthy based on physical examination, baseline blood chemistry, complete blood count, thyroid testing and urinalysis.

Data collection

Dogs were studied in IV (6 mg/kg) and oral (10 mg/kg) pairs.²⁰ For at least six weeks prior to the study, dogs were acclimated to their environment and diet. Immediately prior to the study, random urine samples were collected in all animals for baseline urine citrate concentration and calcium:creatinine ratio. Immediately prior to each study, indwelling external jugular catheters were aseptically placed using manual restraint. Following administration of ZNS,

whole blood samples were collected in lithium heparin Monoject® (Sherwood Medical, St.Louis, Missouri) tubes intermittently for at least three plasma elimination half-lives as determined in a pilot study of two dogs. For the IV study, samples were collected at 0 (before dosing) and at 2.5, 5, 10, 15, 20, 30, 45, 60, 75, 90, 120, 150, 180, 240, 300, 360, 720, 840, 1200, 1440, 1800, and 2160 minutes. Administration of the intravenous solution through a cephalic vein was performed over 10 minutes to ensure the health and safety of the patient. A veterinarian assisted with the monitoring of heart rate, pulse pressure through palpation of femoral pulse, and the mentation of the subjects for any adverse effects from the ethanol. Following a one-week washout, ZNS was administered via the alternative route. For the oral study, sample collection occurred at 0 (before dosing) and at 15, 45, 60, 75, 90, 120, 150, 180, 240, 300, 360, 720, 840, 1200, 1440, 1800, 2160, and 2880 minutes. Urine was collected during IV administration for the first 24 hours to determine the percent and rate of elimination parent ZNS through the renal system via urinary catheter.

Data analysis

Concentrations were quantitated in serum, plasma, whole blood, and urine; refer to chapter II. Drug concentration versus time data for each animal was subjected to standard non-compartmental pharmacokinetic analysis using computer assisted linear regression software WinNonLin® (Pharsight Corporation, Mountain View, California). Area Under the Curve (AUC) was determined to infinity using the linear trapezoidal method; AUC was subsequently used to calculate model independent parameters including mean residence time (MRT) and absolute bioavailability. The extent of absorption was based on absolute bioavailability, F, of the oral product and calculated for each animal from the equation:

$$F = (AUC_{\text{oral}} * Dose_{\text{IV}}) / (AUC_{\text{IV}} * Dose_{\text{oral}}).$$

Rate and extent of absorption was based on estimated peak concentration (C_{\max}) and time to peak (T_{\max}). Duration of exposure to ZNS was based on mean residence time (MRT), and elimination half-life ($t_{1/2}$). From the IV data, V_d , $t_{1/2}$ and clearance (CL) was calculated. All parameters, including RBC accumulation (plasma to whole blood drug concentration), were reported as mean \pm standard deviation. The exception, half-life, was reported as harmonic mean and pseudo-standard deviation. One Way Analysis of Variance (ANOVA) was used to determine if significant differences occurred between each blood compartment. Tukey's (HSD) was used to determine which compartment parameters differ.

Results

Oral administration

Utilizing noncompartmental analysis concentrations of ZNS defined dispositional differences among the tested blood compartments (Fig 2). The $AUC_{(0-\infty)}$ for RBC ($3011.98 \pm$

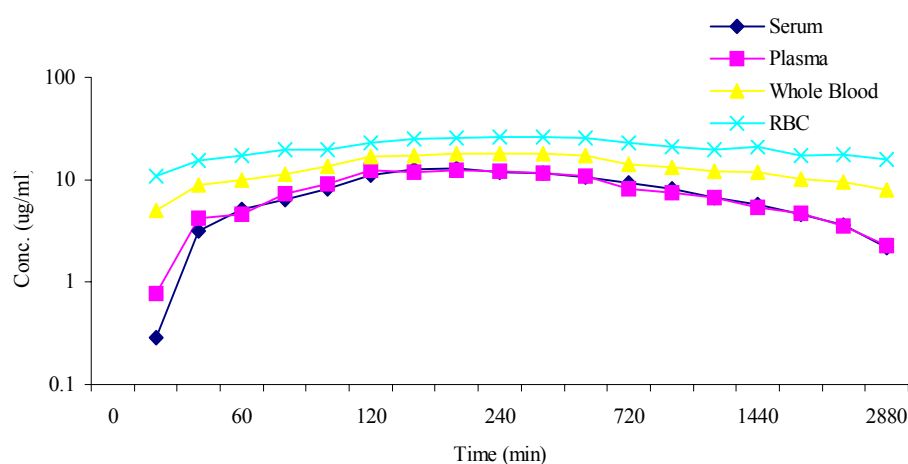


Fig 2. Single oral dose concentrations of ZNS. Data after single oral dose of ZNS ($\mu= 10.25$ mg/kg) plotted logarithmic with calculated concentrations on the y-axis and minutes on the x-axis.

1732.99 hr* $\mu\text{g/ml}$) was greater ($p=0.0001$) than serum (354.47 ± 86.02 hr* $\mu\text{g/ml}$), plasma (346.52 ± 80.24 hr* $\mu\text{g/ml}$) and whole blood (1107.43 ± 315.37 hr* $\mu\text{g/ml}$). The C_{MAX} in serum and plasma of 13.17 ± 1.99 $\mu\text{g/ml}$ and 14.36 ± 2.29 $\mu\text{g/ml}$, respectively, were less than C_{MAX} in RBC (28.74 ± 4.37 $\mu\text{g/ml}$, $p=0.0000$). The concentrations of ZNS in systemic circulation reached T_{MAX} in 2.5 ± 0.65 hr (serum), 2.75 ± 1.25 hr, 3.5 ± 1.04 hr (whole blood) and 4.25 ± 1.33 hr (RBC). RBC time to T_{MAX} was greater ($p=0.0348$) than other blood compartments. The time from administration to absorption of ZNS, T_{lag} , was 0.48 ± 0.3 hr (serum), 0.21 ± 0.29 hr (plasma) 0.04 ± 0.01 hr (whole blood) and 0.04 ± 0.01 hr (RBC) (Table 2).

Table 2. Pharmacokinetic data of ZNS following oral administration. ^a

	Serum (n=8)	Plasma (n=6)	Whole Blood (n=6)	RBC (n=6)	p-value
AUCINF(observed) (hr* $\mu\text{g/mL}$)	354.47 ^A ±86.02	346.52 ^A ±80.24	1107.43 ^A ±315.37	3011.98 ^B ±1733	0.0001
Cmax ($\mu\text{g/ml}$)	13.17 ^A ±1.99	14.36 ^A ±2.29	19.08 ^{A,B} ±2.29	28.74 ^B ±4.37	0.0000
MRT (hr)	26.82 ^A ±6.56	26.89 ^A ±4.6	66.6 ^{A,B} ±23.6	131.56 ^B ±98.9	0.0061
t1/2 (hr)	17.37 ^A ±4.9	17.2 ^A ±3.59	45.80 ^{A,B} ±17.82	90.99 ^B ±70.07	0.0068
Tmax (hr)	2.5 ^A ±0.65	2.75 ^{A,B} ±1.25	3.5 ^{A,B} ±1.04	4.25 ^B ±1.33	0.0348
V _F (ml/kg)	729.11 ^A ±151.9	764.75 ^A ±150	595.49 ^B ±179.48	0.40 ^C ±0.11	0.0000
CL _F (ml/hr/kg)	30.50 ^A ±8.48	31.70 ^A ±7.28	9.55 ^B ±2.83	0.01 ^C ±0	0.0000
Tlag (hr)	048±0.3	0.21±0.29	0.04±0.1	0.04±0.01	
F	163.61	189.63	108.16	126.80	

^a Letters (A,B,C) within each parameter denote different compartments ($\alpha=0.05$, significant when $p < 0.05$) with Tukey's HSD. Parameters AUCINF= Area under the curve measured to infinity observed, Cmax= the maximum concentration, MRT= Mean residue time, t_{1/2}= elimination half life, Tmax= time of maximum concentration, V_F= Volume of distribution, CL_F= Clearance rate, Tlag= Time between administration and absorption, F= absolute bioavailability. See appendix B for equations.

RBC compartment had a greater ($p=0.0061$) MRT (31.56 ± 98.9 hr) than serum (26.82 ± 6.56 hr) and plasma (26.89 ± 4.60 hr). Serum, plasma, and whole blood took 17.37 ± 4.9 hr, 17.2 ± 3.59 hr, and 45.8 ± 17.82 hr, respectively, to eliminate which was less ($p= 0.0000$) than RBC compartment 90.9 hr \pm 70.7 hr. With a single dosage of 6.85 mg/kg IV and 10.25 mg/kg oral, bioavailability was determined to be 163.61 % (serum), 189.63 %, (plasma), 108.16 % (whole blood) and 126.80 % (RBC).

Intravenous administration

Effects presumed to be related to large infusion of ethanol, salivation and ataxia, were not serious and quickly dissipated within 25 minutes of administration.⁴² No other side effects were noted.

Concentrations of ZNS were greater in RBC than the other compartments (Fig 3).

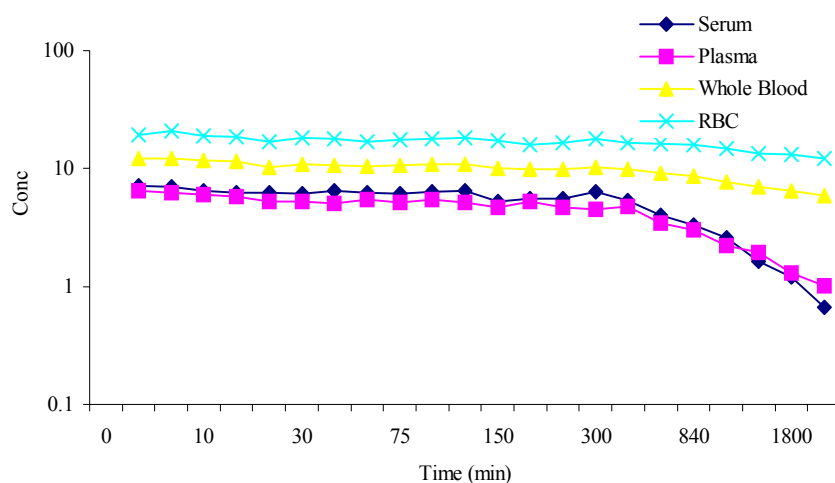


Fig 3. Single dose intravenous concentrations of ZNS. Data after single intravenous dose of ZNS ($\mu= 6.85$ mg/kg) plotted logarithmic with calculated concentrations on the y-axis and minutes on the x-axis.

Maximum concentrations after distribution differed for serum ($8.42 \pm 2.71 \mu\text{g/ml}$), plasma ($6.83 \pm 1.07 \mu\text{g/ml}$), whole blood ($12.58 \pm 1.15 \mu\text{g/ml}$) and RBC ($22.54 \pm 3.98 \mu\text{g/ml}$). The AUC_(0-∞) for RBC ($1587.44 \pm 630.38 \text{ hr} \cdot \mu\text{g/ml}$) and whole blood ($684.23 \pm 256.84 \text{ hr} \cdot \mu\text{g/ml}$) were greater ($p=0.000$) than serum ($144.8 \pm 53.84 \text{ hr} \cdot \mu\text{g/ml}$) and plasma ($122.12 \pm 32.54 \text{ hr} \cdot \mu\text{g/ml}$). The observed Vd of serum ($1193.05 \pm 509.56 \text{ ml/kg}$) and plasma ($1096.05 \pm 251.64 \text{ ml/kg}$) were greater ($p=0.0000$) than whole blood ($673.14 \pm 61.49 \text{ ml/kg}$) and RBC ($379.23 \pm 43.20 \text{ ml/kg}$) (Table 3).

Table 3. Pharmacokinetic data of ZNS following IV administration. ^a

	Serum (n=8)	Plasma (n=6)	Whole Blood (n=6)	RBC (n=6)	p-value
AUCINF(observed) (hr*ug/mL)	144.80 ^A ±53.84	122.12 ^A ±32.54	684.23 ^B ±256.84	1587.44 ^B ±630.38	0.0000
Cl(observed) (mL/hr/kg)	54.62 ^A ±23.29	57.55 ^A ±11.41	11.38 ^B ±2.93	5.06 ^B ±1.83	0.0000
Cmax (observed) (μg/ml)	8.42 ^A ± 2.71	6.83 ^A ± 1.07	12.58 ^B ± 1.15	22.54 ^C ± 3.98	0.0000
MRTINF(observed) (hr)	22.43 ^A ±7.76	19.47 ^A ±4.87	63.33 ^B ±19.6	83.8 ^B ±30.47	0.0000
t1/2 (hr)	16.37 ^A ±7.83	12.9 ^A ±3.58	44.22 ^B ±13.78	57.41 ^B ±21.69	0.0000
Tmax (hr)	0.93 ±1.00	0.44 ±0.54	0.49 ±0.76	0.63 ±0.81	0.8614
Vd(observed) (mL/kg)	1193.05 ^A ± 509.56	1096.05 ^A ± 251.64	673.14 ^B ± 61.49	379.23 ^B ± 43.2	0.0000

^a Letters (A,B,C) within each parameter denote different compartments ($\alpha=0.05$, significant when $p < 0.05$) with Tukey's HSD. Parameters AUCINF= observed Area under the curve measured to infinity, Cl= observed clearance rate, Cmax= the maximum concentration, MRTINF= Mean residue time measured to infinity, $t_{1/2}$ = elimination half life, Tmax= time of maximum concentration, and Vd= Volume of distribution. See appendix B for equations.

Elimination half-life of ZNS was less ($p=0.0000$) in serum ($16.37 \pm 7.83 \text{ hr}$) and plasma ($12.90 \pm 3.58 \text{ hr}$) versus whole blood ($44.22 \pm 13.78 \text{ hr}$) and RBC ($57.41 \pm 21.69 \text{ hr}$). MRT in serum ($22.43 \pm 7.76 \text{ hr}$) and plasma ($19.47 \pm 4.87 \text{ hr}$) were less ($p=0.0000$) than whole blood ($63.33 \pm 19.6 \text{ hr}$) and RBC ($83.80 \pm 30.47 \text{ hr}$). Clearance of ZNS in serum ($54.62 \pm 23.29 \text{ ml/hr/kg}$) and

plasma (57.55 ± 11.41 ml/hr/kg) were greater ($p=0.0000$) than whole blood (11.38 ± 2.93 ml/hr/kg) and RBC (5.06 ± 1.83 ml/hr/kg).

Urine ZNS elimination

The percent recovery of ZNS from urine in the first 24 hours was 5.0 ± 0.34 % . Renal clearance was of 8.73 ± 0.6 $\mu\text{g}/\text{ml}$ (0.0087 ± 0.0 mg/ml). (Table 4).

Table 4. Urine ZNS recovery and elimination rate.^a

	Mean	Std. Dev
% Recovered of ZNS drug in Urine	5.00	± 0.34
Renal clearance rate (mg/ min)	0.009	± 0.00
Renal clearance rate ($\mu\text{g}/\text{min}$)	8.73	± 0.60

^aThe percent (%) recovery and renal clearance following single IV dosing in 3 apparently healthy hound dogs receiving zonisamide (6.85 mg/kg) IV

Discussion

Single dose administration was used to establish a model for development of long-term therapy through the establishment of the disposition of ZNS. Studies done previously were performed for purposes of human implementation; however, limited data for the dogs does exist. In comparing the canine results among investigators, , elimination half-lives were similar. Plasma and RBC times previously reported were 14 and 42 hours, respectively, following a 20 mg/kg dose of ZNS¹⁰ compared to 16.9 (plasma) and 58.01 (RBC) hours in this study. Elimination half-life of ZNS is greater in humans, ranging among investigators between 52 and 60 hrs^{8,14} in plasma. AUC in plasma was observed at $486 \text{ hr} \cdot \mu\text{g}/\text{ml}$ ¹⁰ comparing to 346.52

hr* μ g/ml in this study. The Vd was 0.72 L/kg in plasma compared to 0.9 L/kg for dogs reported in data released from the manufacturer's laboratory.¹⁰ Compared to humans (1.27 L/kg)¹⁴ receiving ZNS, Vd in dogs was less. The amount of time to reach maximum plasma concentrations in humans (4-6 hrs)¹⁴ was greater than observed in this study (2.75 hrs).

The concentration of ZNS differed among the blood compartments following oral administration. Data reflected C_{\max} in RBC greater than other compartments. The $AUC_{(0-\infty)}$ and elimination half-life were greater for RBC corresponding to a MRT 131.56 hours. RBC values were different from data collected in serum and plasma. The calculated parameters for serum and plasma showed no differences statistically in the data observed between the compartments. Whole blood differed from all compartments in the apparent Vd observed (595.49 ml/kg) and Cl (9.55 ml/hr/kg). Determination of bioavailability in noncompartmental analysis revealed values ranging from 108.16-189.63 % were greater than the expected human values (84%).⁸ Intravenous observations noted similar differences between RBC and serum as in oral administration. Values seem consistent with RBC having greater concentration and elimination rates that are longer than other compartments.

The apparent reason for RBC differences is due to binding of ZNS to RBC.¹¹ The pharmacologic activity of ZNS is extended as seen in elimination half-life because of the uptake and confinement of ZNS.¹¹ The noted differences observed in concentrations across the same time point can be due to the analysis of serum and plasma versus whole blood. With the absence of RBC in serum and plasma, the concentrations at those time points are lower. Analysis of the data revealed elevated bioavailability that need to be explained. The equation for bioavailability (appendix B) uses the data collected from $AUC_{(0-\infty)}$ from IV and oral administration. Possible reasons for the increased value were explored in the calculation of $AUC_{(0-\infty)}$. The kinetic software calculates Cl using Dose/ $AUC_{(0-\infty)}$. Considering that $AUC_{(0-\infty)}$ and Cl are inversely

proportionally, changes to one affect the other inversely. The use of ethanol as a vehicle for IV administration may have contributed to bioavailability that exceeded 100%. Ethanol can increase renal blood flow and urine output^{43,44} and, presumably, the elimination of ZNS. Ethanol causes hepatic saturation of microsomal enzymes leading to longer exposure of ethanol when large doses of ethanol are administered.⁴³ Ethanol also is reported to induce cytochrome P450 isozyme 3A4 (CYP 3A4)⁴⁵ the microsomal enzyme responsible for metabolism in humans.¹⁷ Due to ethanol saturation of microsomal enzymes and the induction of CYP 3A4 and extensive renal elimination of ZNS a possible reason for the to the high bioavailability is due to the effects of ethanol on increasing clearance which inversely lowers the $AUC_{(0-\infty)}$ by decreasing ZNS concentrations in vivo. However, the extent of such evidence is hypothesized, due to the unlikely event that induction of the CYP3A4 could occur quickly based on the single injection of ethanol and the time it would take in vivo to cause induction.

In developing a regimen for multiple dosing, the steady state concentrations in the dogs would be attained between 4 to 7 days for plasma and whole blood, respectively. These estimations are based on 97 - 99 % of steady state concentrations being reached in 5.5 half-lives.⁴⁶ With an anticipated fluctuation of 14% following twice daily dosing⁸, a dose of 10 mg/kg (~300 mg) was used to achieve therapeutic levels suspected in humans ranging from 16.5 to 49.6µg/ml.⁹ The length of time estimated to maintain therapeutic levels was considered acceptable due to the relatively small fluctuation ensuring that concentrations did not fall below what was considered to be the window. The accumulation of ZNS within the dog was determined to be 2.02 in plasma and 4.09 in whole blood data collected if administered twice daily. Anticipation of higher concentrations because of the accumulation index provides additional support to the maintenance of therapeutic levels which was observed in multiple dosings.

In conclusion, ZNS appears to be eliminated by means other than renal excretion of the parent compound. Based on the results of this study, a dose of 10 mg/kg is recommended every 12 hrs in order to achieve and maintain effective concentrations. Because tissue concentrations significantly differed following single dosing, monitoring methods might best be determined following multiple dosing.

CHAPTER IV

MULTIPLE DOSE PHARMACOKINETIC DATA

Introduction

Multiple dose studies were performed to verify that the dosing regimen derived from the single dose study would maintain therapeutics concentrations and monitor the fluctuation between twice daily dosings. The data collected assisted in the development of a long-term regiment by recommending dosages and frequency of dosing. Safety was evaluated based on clinical pathology and thyroid testing with a focus on organs of excretion.

Materials and Methods

Data collection

Eight apparently healthy dogs (used in single-dose studies) were studied. Zonisamide was administered orally every 12 hours at 10 mg/kg. Blood samples and urine were collected prior to dosing to determine baseline for clinical laboratory tests, urine and thyroid hormone analysis. Peak and trough blood samples were collected (based on the single-dose study) and once a week for eight weeks beginning with the first dose. Clean catch urine samples were collected at each trough sampling time. After the final dose of ZNS at the end of the eight weeks, blood was collected at 0 (time of last dose) and 1.5, 3, 16, 28, 48, 72 hours. At the end of the dosing period, clinical laboratory tests, determined through automated assays validated in the dog, urine and thyroid hormone analysis were repeated. Thyroxin-4 (T4), free and total were analyzed through equilibrium dialysis and thyroid-stimulating hormone (TSH) was evaluated through automated chemiluminescence. Citrate levels were measured through ion chromatography.

Data analysis

Samples were analyzed using methods described in chapter II. From the chronic dosing data, non-compartmental analysis was used to determine $AUC_{(0-\infty)}$, percent fluctuation, accumulation, and elimination half-life. Peak drug concentration (C_{max}) and minimum concentrations was evaluated and accumulation was determined. One-way ANOVA was used to determine if any differences occurred between the blood compartments (serum, plasma, whole blood, and RBC) and to assist with determining disposition and therapeutic monitoring. Tukey's (HSD) was used to determine which compartment parameters differ.

Clinical laboratory tests and urinary calcium: creatinine ratios and urine citrate concentrations were compared to a baseline at the study's end using a paired Student's t test. Level of significance for all values was if p-value is less than $\alpha=0.05$.

Results

Concentrations of ZNS appeared to attain steady state by day 7 in all analyzed blood compartments (Fig 4). Maximum concentrations of ZNS following administration averaged 52.45 ug/ml (serum), 56.01 μ g/ml (plasma), 55.56 μ g/ml (whole blood), and 55.19 μ g/ml (RBC) among tested dogs (Table 5). The percent fluctuation between dosing for steady state between

Table 5. Maximum and minimum concentrations of multiple dosings of ZNS.^a

	Max (μ g/ml)	Min (μ g/ml)	Fluctuation (%)
Serum	52.45	45.32	13.6
Plasma	56.01	50.37	10.0
Whole Blood	55.56	50.43	9.2
RBC	55.19	50.57	8.4
Mean	54.8 \pm 1.6	49.17 \pm 2.57	10.3 \pm 2.3

^a Data is an average maximum and minimum concentration of the analyzed blood compartments for the 8 dogs after receiving 10.17mg/kg oral for 8 weeks.

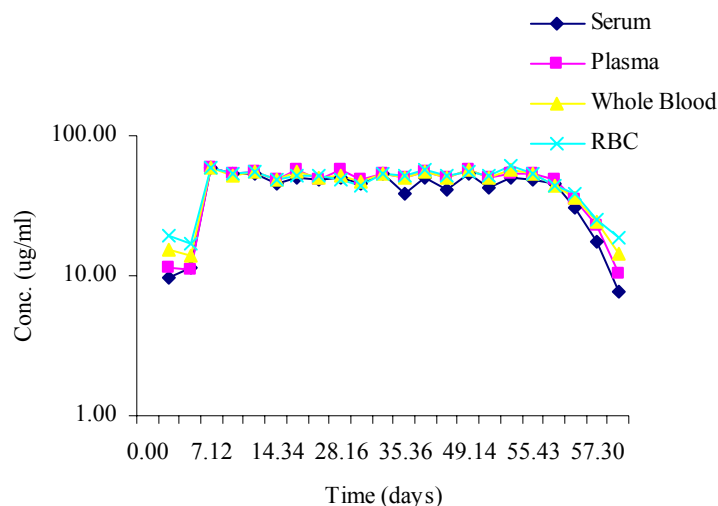


Fig 4. Multiple dosing concentrations of ZNS. Data collected following 10.17 mg/kg dose for 8 healthy dogs for 8 weeks followed by 72 hours of elimination data analysis.

C_{max} and C_{min} was calculated to be 13.6 % (serum), 10 % (plasma), 9.2 % (whole blood), and 8.4 % (RBC). The AUC_(0-∞) determined from the terminal period of the study in serum (2339.2 ± 851.28 hr*µg/ml), plasma (2661.6 ± 934.22 hr*µg/ml), whole blood (2990.64 ± 599.76 hr*µg/ml), and RBC (3683.04 ± 1008 hr*µg/ml) were different (p=0.001). Accumulation index of ZNSI was , in the respective tissues, serum (3.26 ± 0.69), plasma (3.52 ± 0.68), whole blood (4.30 ± 0.56), and RBC (5.46 ± 1.7). The half-life of ZNS following its discontinuation was greater(p= 0.001) in RBC (37.2 ± 12.24 hr) and whole blood (30.9 ± 4.25 hr) compared to serum (21.4 ± 5.38 hr) and plasma (23.52 ± 5.76). (Table 6)

Safety

Clinical pathology

Differences in clinical pathology data at the study's end compared to baselines were

Table 6. Pharmacokinetic data of ZNS following multiple dosing oral administration.^a

	Serum (n=8)	Plasma (n=8)	Whole Blood (n=8)	RBC (n=8)	p-value
Accumulation Index	3.26 ^A ± 0.69	3.52 ^A ± 0.69	4.30 ^B ± 0.56	5.46 ^C ± 1.7	0.001
AUCINF(observed) (hr*ug/mL)	2239.2 ^A ± 851.28	2661.6 ^B ± 934.32	2990.64 ^C ± 599.76	3683.04 ^D ± 1008	0.001
Lambda_z (1/hr)	0.03 ^A ± 0.01	0.03 ^B ± 0.01	0.02 ^C ± 0.0	0.02 ^D ± 0.01	0.001
t 1/2 (hr)	21.4 ^A ± 5.38	23.52 ^A ± 5.76	30.96 ^B ± 4.32	37.2 ^C ± 12.24	0.001

^a Data following administration of oral ZNS (10.17 mg/kg) for 8 weeks, n=8. Parameters are calculated from the terminal (elimination) portion after discontinuation of the drug. Letters (A,B,C,D) within each parameter denote different compartments ($\alpha=0.05$, significant when $p < 0.05$) with Tukey's HSD. Parameters AUCINF= observed Area under the curve measured to infinity, Lambda_z= elimination constant, and t 1/2= elimination half life. See appendix B for equations.

reported in several of the tested parameters, although all values were within normal reference ranges before and at study end. Differences seen in serum alkaline phosphatase ($p = 0.0464$) and serum calcium ($p = 0.004$) were greater than baseline. Serum total protein ($p = 0.0071$), including albumin ($p = 0.0266$), were both less than the baseline (Table 7).

Thyroid testing

Tests indicative of thyroid function differed across time. Total T4 was less at study end compared to baseline and decreased to below normal limits by the end of the dosing period. Differences in free T4 and TSH from baseline were observed (Table 8).

Urinary testing

Urine citrate ($p = 0.0098$) and calcium ($p = 0.214$) concentrations were greater at study end compared to baseline whereas urinary creatinine concentrations ($p = 0.081$) and the calcium:creatinine ratio ($p = 0.2873$) were both less than baseline (Table 9).

Table 7. Complete blood count and chemistry panel. ^a

Parameter	reference range	Initial	Std. dev	End	Std. dev	P-value
WBC (x 10 ³ /ml)	6000-17000	7.21	1.82	10.90	3.72	0.1278
RBC (x 10 ³ /ml)	5.5-8.5	7.25	0.33	6.91	0.13	0.0151
Hgb (g/dl)	12-18	16.60	0.67	15.93	0.15	0.0131
PCV (%)	37-55	50.10	1.93	47.46	1.30	0.0172
MCV (fl.)	60-77	69.19	1.85	68.70	2.47	0.0022
MCHC (g/dl)	32-36	33.13	0.50	34.40	0.44	0.0120
Plasma protein (g/dl)	6-8	7.10	0.35	6.83	0.21	0.0122
Glucose (mg/dl)	60-135	69.86	5.87	81.57	16.26	0.0491
Cholesterol (mg/dl)	120-247	182.29	47.96	223.29	56.25	0.0641
BUN (mg/dl)	5.0-29	13.29	5.38	21.00	5.26	0.1409
Creatinine (mg/dl)	0.3-2	0.96	0.13	1.13	0.18	0.0524
Magnesium (mg/dl)	1.7-2.1	2.09	0.18	2.11	0.16	0.0042
Calcium (mg/dl)	9.3-11.8	9.97	0.52	10.11	0.37	0.0045
Phosphorus (mg/dl)	2.9-6.2	3.74	0.36	4.41	0.72	0.0523
Total Protein (g/dl)	5.7-7.8	6.49	0.37	6.34	0.40	0.0071
Albumin (g/dl)	2.4-3.6	3.20	0.14	2.94	0.28	0.0266
Globulin (g/dl)	1.7-3.8	3.29	0.27	3.40	0.14	0.0109
ALT (u/L)	10-130	41.71	6.97	34.71	8.75	0.0581
ALKP (u/L)	24-147	77.00	21.70	89.14	41.56	0.0464
GGT (u/L)	0-25	12.86	1.95	12.71	2.87	0.0036
Total Bilirubin (mg/dl)	0-0.8	0.12	0.04	0.20	0.00	0.1560

^a Data reported as a baseline before and after chronic administration of 10.17 mg/kg of ZNS to 8 dogs. Significant if $p < \alpha = 0.05$. Parameters are WBC= white blood cells, RBC= red blood cell, Hgb= hemoglobin, PCV= packed cell volume, MCV= mean corpuscular volume, MCHC= mean corpuscular hemoglobin concentration, BUN= blood urea nitrogen, ALT= alanine aminotransferase, ALKP= alkaline phosphatase, and GGT= gamma glutamyltransferase

Table 8. Thyroid hormone concentrations. ^a

Thyroid		Baseline	8 weeks	P-value
		Mean	Mean	
Free T4	<i>0.07-3.1 ng/dl</i>	1.29±0.27	1.08±0.35	0.0546
Total T4	<i>1.61-3.6 ug/dl</i>	1.74±0.57	1.05±0.43	0.0514
TSH	<i>0-0.32 ng/dl</i>	0.18±0.2	0.21±0.17	0.1516

^a Thyroid hormones were evaluated and compared from beginning of the chronic dose period to the end of the 8 weeks. Student test was used to signify a difference in means. $\alpha = 0.05$. n=8

Table 9. Urine citrate, calcium, creatinine, and calcium: creatinine ratio. ^a

Measurement		Baseline		8 weeks		P value
Urine	Reference	Mean	Std Dev	Mean	Std Dev	
Calcium	<i>mg/dl</i>	1.49	0.45	3.08	3.59	0.214
Creatinine	<i>mg/dl</i>	259.48	70.58	200.62	37.91	0.081
Ratio		0.0059	0.0019	0.0171	0.023	0.2873
Citrate	<i>g/ml</i>	44.7	4.32	46.1	7.64	0.0098

^aThe data was reported in 5 apparently healthy hound dogs receiving zonisamide (10.17 mg/kg twice daily) orally for the 8 week chronic study.

Discussion

Overall, the effects of multiple dosing indicate that little difference in the kinetics of ZNS among the different blood compartments as compared to differences seen following single administrations due to RBC. Data collected indicates that equilibrium was reached in all compartments by steady state. With sample collection performed once weekly, by day 7 the dogs had reached a level that would be consistent with steady state and this corresponds to previous estimates of time in chapter III.⁴⁶ The amount of fluctuation determined from data was 10.3 ± 2.3 % for serum, plasma, whole blood, and RBC on average. This corresponds to fluctuations of 14 % following twice daily dosing in humans.⁸ The amount of fluctuation was used to ensure that dosages given maintained the appropriate benefit for therapy. As mentioned in chapter III, the accumulation of plasma and whole blood were expected to be 2.02 and 4.09, respectively. Data collected supports this estimate with plasma (3.5 ± 0.68) and whole blood (4.3 ± 0.56) being similar. With the accumulation of ZNS being great, a reason may be that the dosing interval is too short. However, since the elimination half-life is 21.4 to 30.9 hours in plasma and whole blood respectively, twice daily dosing is acceptable with the presence of minor fluctuations in

steady state concentrations.⁴⁷ The predicted accumulation index for plasma (2.02) and whole blood (4.09) were actually 3.52 for plasma and 4.3 for whole blood.

The differences observed in data analysis reveals that the changes in disposition of ZNS following single administration differ with interest to the role of RBC. The differences could be reflect reversible uptake of ZNS by erythrocytes.¹¹ Once RBC saturation occurred following multiple dosing, blood compartments equilibrated and the concentrations and kinetic data collected were similar as seen by the figure 4. This equilibration across compartments provides evidence that the concentration of ZNS in each of the other compartments can predict total systemic concentrations.

All dogs tolerated oral ZNS well. Limited reports of diarrhea occurred and were determined to be unrelated to the administration of ZNS due to the diarrhea subsiding without changes in the dose or dosing interval. Routine blood work over the course of the chronic study was analyzed and all parameters were found to be within normal reference ranges. Variations seen in parameters were concluded to be due to daily physiologic changes and could not be attributed to affects of ZNS on any one system.

Sulfonamide drugs are recognized to negatively impact thyroid function testing in dogs. Impairment of thyroglobulin and tyrosine coupling decrease the amount of active thyroid hormone produced.^{27,48} However, total T4, by the end of the study, was lower and indicated possible hypothyroidism. Values for free T4 (lower) and TSH (higher), although within normal range, were indicative of possible changes to the thyroid function.. Increases in TSH are attributed to low levels of thyroid hormone negatively inhibiting and slowing the release of TSH.⁴⁸ As circulating levels of thyroid hormones decrease potential problems related hypothyroidism: metabolic and gastrointestinal problems can occur.⁴⁸

The carbonic anhydrase inhibitory activity of ZNS may contribute to urolithiasis (calcium phosphate or calcium oxalate) by increasing calcium excretion and lowering citrate in the urine.^{11,24} Higher levels of calcium were found in urine samples by study's end. In response to this, the calcium:creatinine ratio was higher which has been previously observed with ZNS administration in the development of uroliths.²⁵ Citrate levels were greater by the end, which contraindicates the expected result following the administration of a carbonic anhydrase inhibitor. Possible reasons could be attributed to problems related to storage or handling of urine prior to analysis. Overall, the effects of ZNS on the urinary system seem to be at a minimum, but continued evaluation of these parameters would be justified with a urinalysis to determine any formation of uroliths.

Considering the disposition of ZNS following multiple dosing, determination was made that monitoring ZNS concentrations in plasma, serum, and whole blood would provide data sufficient to predict the total amount of ZNS within a patient. The amount of fluctuation and favorable elimination half-life offers evidence that a dose of 10 mg/kg dosed twice daily would ensure that concentrations, considered therapeutic in humans, can be maintained. Evaluation of clinical pathology, thyroid testing, and urine analysis would need to be performed to monitor any changes which may be to the detriment of the patient.

CHAPTER V

SUMMARY AND CONCLUSION

Summary

Idiopathic epilepsy in the dog is a difficult disease to treat; long-term use of the most commonly selected anticonvulsants have associated problems. Data collected through the single and multiple dose administration provided evidence that kinetic data collected compare to the limited canine research of ZNS disposition.

Following single administration, differences observed in the interaction between RBC and ZNS were pivotal in explaining the differences that were observed in the disposition of the ZNS. The half life was 90.9 hr and 57.4 hr following oral and IV administration, respectively, for RBC, compared to half-lives of 17.2 hr and 12.9 hr following oral and IV administration, respectively, in plasma. The $AUC_{(0-\infty)}$ was greater in RBC for both oral and IV data compared to the other blood compartments. A MRT of 131.5 hrs in RBC was observed compared to 26.8 hrs for both serum and plasma following oral administration. The MRT for IV dosing was greater in RBC than the other compartments. The Vd of ZNS following IV administration was 1193.1 ml/kg for serum, 1096.1 ml/kg for plasma, 673.1 ml/kg for whole blood, and 379.2 ml/kg for RBC. Except for RBC, the Vd was indicative of the drug's ability to move throughout the total body water of the dog. Observed parameters for both oral and IV dosing revealed that the RBC parameter on a whole was different than the other blood compartments.

The changes that were observed due to the effects of RBC following single dosing were absent in multiple dosing, presumably due to the saturation and reversible binding with aforementioned RBC.¹¹ A favorable elimination half-life ensures that elimination of the drug would not be less than the dosing period which would aide in providing therapeutic benefit to patients. The half-life observed ranged from 21.4 hrs to 37.2 hrs for serum and RBC,

respectively. The favorable half-life of plasma is important because of plasma's ability to carry free ZNS to brain tissue and interact with tissues to cease induction of seizures. Due to the movement of plasma and the interactions with RBC monitoring plasma concentrations would be sufficient to predict total ZNS concentrations. Accumulation of ZNS in all blood compartments was observed and ranged from 3.2 to 5.4 (C_{max} following first dose compared to C_{max} at last dose). Based on human studies, ZNS concentrations at steady state when receiving 200mg twice daily had minor fluctuation (13.5 %) and obtained steady state by day 29.⁸ The therapeutic level suggested for humans in the previous statement was 15-25 µg/ml. In the dog, ZNS had small fluctuations (μ = 10.3 %) during the dosing interval following multiple administration. This small fluctuation supported the administration of ZNS twice daily (every 12 hours) as seen in humans. Since small differences were observed in multiple dosing and saturation of RBC occurred, favorable long-term monitoring of plasma and a recommended dose of 10 mg/kg can be implemented..

Minor changes in clinical pathology were observed, but all results remained within normal ranges. Despite normalcy throughout this 8 week study period, metabolism of ZNS, by microsomal enzymes of phase I and conjugation of phase II, indicate hepatic function be monitored intermittently until sufficient number of animals have been studied to indicate safety.

Urinary calcium concentrations and calcium: creatinine urine ratios were greater at study end compared to the beginning. This finding may indicate potential formation of urinary calculi, and, if unmonitored, this might lead to the formation of uroliths. Monitoring the formation of urinary calculi by evaluating the sediment following urinalysis could be performed to monitor crystal development. However, the observed urinary citrate concentration was greater by study end. This unexpected finding indicates that possibly the movement of citrate in the renal structure is impaired and may account for the increased levels of citrate in urine. Changes in the

urine (calcium, calcium: creatinine ratio, and citrate) should to be monitored during clinical trials to detect the potential for development of uroliths due to the carbonic anhydrase inhibitory activity of ZNS.

Changes in the thyroid hormone (T4) were observed due to the effects of sulfonamides²⁶ and the disruption of the TSH/ thyroid relationship. Thyroid changes were indicative of possible hypothyroidism. Total T4 and free T4 were lower at study's end while TSH was elevated. Although the values were insignificant the changes indicate that they need to be monitored in long-term therapy to provide evidence based on a larger population to make a determination of whether ZNS is goitrogenic.

All the data collected was imperative in establishing ZNS disposition and safety parameters to consider when implementing and modifying a dosing regimen.

Conclusion

In conclusion, a twice daily dose of 10 mg/kg would be sufficient to maintain expected therapeutic concentrations. Therapeutic drug monitoring of either serum or plasma would be used to accurately predict the concentration of ZNS in systemic circulation. Routine blood work and thyroid hormone testing should be performed to note any changes related to administration of ZNS. Urolith formation should be monitored by urinalysis looking for urine crystals and through the analysis of calcium, calcium: creatinine ratio and microscopic examination for crystals (calcium phosphate or calcium oxalate). A pharmacodynamic study would not only help with establishing the therapeutic range for control of idiopathic epilepsy, but also provide a better representation for potential side effects not evident in this study.

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APPENDIX A

Ethanol concentrations

Table 10. Ethanol concentrations related to ethylene glycol treatment dose and LD100.

Dog	Ethanol received		Ethylene glycol toxicity (5 ml/kg)		LD100 (5.365g/kg)
	(ml)	(g/kg)	(ml)	(g)	(g)
1	23.35	0.20	209.55	204.41	
2	19.98	0.20	179.30	174.90	
3	21.08	0.20	189.20	184.56	
4	12.96	0.20	117.70	114.81	
5	16.43	0.20	151.25	147.54	
6	14.76	0.18	150.70	147.00	
7	17.07	0.20	158.40	154.51	
8	15.01	0.20	138.05	134.66	

The amount of ethanol administered intravenous to each dog over the course of 10 minutes compared to the dogs recommended dose for ethylene glycol toxicities which is 20% ethanol infusion 4 times every 6 hours³⁷ and the lethal dose of ethanol when administered intravenously.³⁸

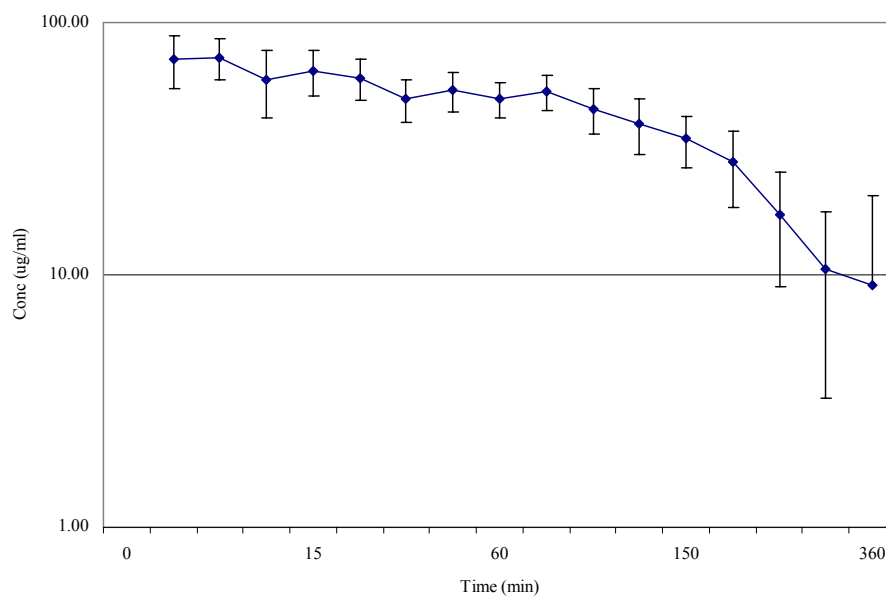


Fig 5. Ethanol concentration for IV administration. Ethanol concentrations after intravenous infusion of ZNS over 360 minutes. Infusion time was 10 minutes. Standard deviation for each concentration plotted. n=8.

APPENDIX B

Equations

WinNonLin[®] Pharmacokinetic equations

$$\text{Accumulation index} = \frac{1}{[1 - e^{-(\lambda_z - \tau)}]}$$

where λ_z = first order rate constant related to elimination and τ = dosing interval

AUC: Area under the curve measured with linear trapezoidal method

$$= AUC|_{t_1}^{t_2} = \delta t * \frac{C_1 + C_2}{2}$$

AUCINF: AUC from the time of dosing extrapolated to infinity.

$$= AUC_{last} + \frac{C_{last}}{\lambda_z}$$

AUMCINF: Area under the moment extrapolated to infinity

$$= AUMC_{last} + \frac{t_{last} * C_{last}}{\lambda_z} + \frac{C_{last}}{\lambda_z^2}$$

Cl_{ss}, Cl_{ss}/F: for steady state data is the estimate of total body clearance where F is the fraction absorbed

$$= \frac{\text{Dose}}{AUC_0^\tau}$$

$$= \text{DOSE} / \text{AUCINF}$$

Lambda z (λ_z): First order rate constant associated with the terminal (log-linear) portion of the curve.

t^{1/2} Lambda z: Terminal half-life

$$= \frac{\ln(2)}{\lambda_z}$$

MRTINF: mean residence time (MRT) extrapolated to infinity for non-infusion model

$$= \frac{AUMCINF}{AUCINF}$$

for infusion model

$$= \frac{AUMCINF}{AUCINF} - \frac{TI}{2} \quad \text{where TI is the length of infusion}$$

V_z, V_z/F: volume of distribution based on the terminal phase

$$= \frac{DOSE}{\lambda_z * AUCINF}$$

% Fluctuation: Fluctuation for steady state where max and min fall within the dosing interval

$$= \frac{C_{\max} - C_{\min}}{C_{\text{avg}}} * 100$$

Other equations

Protein Binding:
$$= \frac{[Plasma] - [Buffer]}{Plasma} * 100$$

Relative bioavailability (F):

$$F = \frac{(AUC_{\text{Oral}} * Dose_{\text{IV}})}{AUC_{\text{IV}} * Dose_{\text{Oral}}}$$

RBC Concentration:
$$= \frac{1}{H} [C_B - (1-H)C_P]$$
 where

RBC= Red Blood Cell

H= Fractional hematocrit

C_B = Whole Blood concentration of zonisamide

C_P= plasma concentration of zonisamide

APPENDIX C

Controls

Table 11. Intra-assay controls for serum, plasma, and whole blood.^a

serum	2	5	7	15	18	20	25	30	60
mean (µg/ml)	2.12	4.64	6.50	13.76	16.98	18.27	23.51	30.09	57.56
std dev	0.33	0.29	0.91	1.68	1.12	0.48	2.09	1.51	4.79
precision error	15.57	6.34	14.00	12.21	6.60	2.64	8.91	5.03	8.32
% Accuracy	106.02	92.88	92.86	91.73	94.36	91.33	94.03	100.31	95.93

Serum % accuracy= 95.5 ± 4.78 %

plasma	3	7	15	20	25	45	70	75	90
mean (µg/ml)	2.64	6.79	14.10	19.98	24.15	43.45	68.72	73.29	99.96
std dev	0.36	0.48	0.95	0.57	2.43	2.87	3.46	10.23	11.07
precision error	13.61	7.04	6.71	2.84	10.05	6.59	5.03	13.96	11.07
% Accuracy	87.96	96.98	94.01	99.90	96.58	96.56	98.17	97.72	111.07

Plasma % accuracy= 97.7 ± 6.08 %

whole blood	1	3	7	15	30	45	70	90
mean (µg/ml)	0.95	2.56	5.88	14.66	29.35	41.33	63.09	90.67
std dev	0.18	0.25	0.23	0.95	1.69	3.97	1.48	1.40
precision error	19.31	9.79	3.96	6.47	5.74	9.61	2.35	1.54
% Accuracy	94.66	85.31	84.06	97.73	97.84	91.85	90.13	100.75

Whole blood % accuracy= 92.8 ± 6.06%

^a Controls (µg/ml) of selected compartments and the respective % accuracy with mean and standard deviation for each compartment compared against known concentrations of ZNS. Variability of ± 15 % was acceptable for controls in this study.

Table 12. Inter-assay controls for serum, plasma, whole blood, and urine.^a

Serum	2	5	7	15	18	20	25	30	60
mean (µg/ml)	1.91	4.89	7.41	14.80	17.75	18.90	24.82	30.93	61.31
std dev	0.07	0.01	0.00	1.10	0.81	0.42	0.34	0.63	2.17
precision error	3.90	0.28	0.05	7.46	4.59	2.22	1.37	2.02	3.54
% Accuracy	95.41	97.75	105.84	98.64	98.63	94.52	99.28	103.10	102.19

Serum % precision = 99.5 ± 3.7 %

Plasma	3	7	15	20	25	45	70	75	90
mean (µg/ml)	3.07	6.75	13.88	19.65	23.05	44.20	71.02	78.05	98.83
std dev	0.18	0.65	0.20	0.67	0.45	1.37	0.87	8.60	11.70
precision error	5.70	9.56	1.47	3.41	1.95	3.09	1.22	11.02	11.84
% Accuracy	102.47	96.47	92.55	98.24	92.19	98.23	101.46	104.06	109.81

Plasma % precision = 99.5 ± 5.6 %

Whole Blood controls	1	3	7	15	30	45	70	90
mean (µg/ml)	0.98	2.62	6.500	15.23	30.32	43.89	64.45	89.51
std dev	0.03	0.15	0.233	0.29	0.13	3.35	0.17	1.25
precision error	3.53	5.77	3.964	1.87	0.43	7.64	0.27	1.39
% Accuracy	98.04	87.48	84.061	101.50	101.08	97.54	92.08	99.45

Whole blood % precision = 95.2 ± 6.5 %

urine	15	45	70	90
mean (µg/ml)	16.96	42.77	75.79	86.77
std dev	0.69	2.04	0.60	1.85
precision error	4.06	4.77	0.79	2.14
% Accuracy	113.05	95.05	108.27	96.41

Urine %precision = 103.2 ± 8.86 %

^a Controls (µg/ml) of selected compartments and the respective %precision with mean and standard deviation for each compartment compared against known concentrations of ZNS. Variability of ± 15 % was acceptable for controls in this study.

APPENDIX D
Compartmental Data

Table 13. Compartmental oral pharmacokinetic data of ZNS. ^a

	Serum (n=6)	Plasma (n=6)	Whole Blood (n=6)	RBC (n=6)	p-value
AUCINF (hr*ug/mL)	358.14 ^A ±89.23	337.08 ^A ±85.33	912.53 ^B ±213.47	2199.70 ^C ±501.8	0.0000
Vd_F (mL/kg)	753.92 ^A ± 90.86	727.44 ^A ± 136.22	525.21 ^B ± 72.47	0.38 ^C ± 0.05	0.0000
CL_F (mL/hr/kg)	30.36 ^A ±9.10	31.19 ^A ±8.83	11.47 ^B ±3.34	0.01 ^B ±0	0.0000
Cmax (µg/ml)	12.56 ^A ± 1.80	12.58 ^A ± 1.89	17.81 ^B ± 1.41	25.27 ^C ± 1.89	0.0000
α (1/hr)	2.34 ±1.95	2.18 ±1.51	1.81 ±1.31	2.38 ±2.70	0.8855
α half life (hr)	0.42 ±0.19	0.44 ±0.28	0.51 ±0.24	0.51 ±0.29	0.7475
β (1/hr)	0.04 ^A ±0.01	0.04 ^A ±0.01	0.02 ^B ±0.01	0.01 ^B ±0.00	0.0000
β half life (hr)	18.18 ^A ±4.31	16.90 ^A ±4.26	33.12 ^B ±6.8	58.01 ^C ±13.41	0.0000
Tmax (hr)	2.97 ±0.91	2.89 ±1.06	3.33 ±1.13	3.49 ±1.58	0.7446
MAT (hr)	2.34 ± 3.79	2.18 ± 2.27	1.81 ± 1.72	2.38 ± 7.3	0.9524
Tlag (hr)	0.73 ±0.44	0.64 ±0.52	0.35 ±0.47	0.12 ±0.34	
F	196.85	89.65	98.45	67.52	

^a Letters (A,B,C) within each parameter denote different compartments ($\alpha=0.05$, significant when $p < 0.05$) with Tukey's HSD. Parameters AUCINF= Area under the curve measured to infinity observed, Vd_F= Volume of distribution, CL_F= systemic clearance rate, Cmax= the maximum concentration, α and β are the absorption and elimination rate constant, respectively, α and β are the absorption and elimination half lives, respectively, Tmax= time of maximum concentration, MAT= Mean absorption time, Tlag= Time between administration and absorption, F= absolute bioavailability. See appendix B for equations.

Compartmental analysis was performed on the collected ZNS samples to provide parameters that noncompartmental analysis could not provide such as absorption and elimination rate constants and extrapolated values such as A and B. After reviewing the data, determination

was made not to use the compartmental data because of the small number of acceptable data points that were accepted for stripping using the kinetic software. Some of the data collected contradicted the definition of the parameter, such as $A < B$ where A, related to absorption, and B, related to elimination, should be $B > A$. Because of the unexplainable results determination was made not to use this data. The collected data for both oral and IV administration are listed below to review.

Table 14. Compartmental IV pharmacokinetic data of ZNS. ^a

	Serum (n=4)	Plasma (n=5)	Whole Blood (n=4)	RBC (n=5)	p-value
A ug/mL	0.84 ± 1.08	2.61 ± 3.86	2.15 ± 1.73	1.24 ± 2.24	0.7910
α (1/hr)	0.30 ±0.31	0.04 ±0.01	0.02 ±0.01	0.11 ±0.16	0.1067
α -HL (hr)	9.29 ^A ±9.29	23.69 ^{A,B} ±13.16	41.52 ^B ±10.96	18.75 ^{A,B} ±13.36	0.0136
B ug/mL	35.11 ^A ± 6.74	38.02 ^A ± 5.94	69.46 ^A ± 3.71	120.9 ^C ± 11.38	0.0000
β (1/hr)	0.042 ^A ±0.01	0.036 ^{A,B} ±0.02	0.018 ^{B,C} ±0.00	0.006 ^C ±0.00	0.0004
β -HL (hr)	16.55 ^A ±2.15	29.87 ^A ±27.26	43.31 ^A ±7.41	88.92 ^B ±16.35	0.0001
AUCINF (hr*ug/mL)	121.58 ^A ±38.38	251.28 ^A ±254.68	619.41 ^A ±81.39	2177.09 ^B ±523.47	0.0000
CL (mL/hr/kg)	8.88 ^A ±2.88	6.48 ^A ±3.40	1.63 ^B ±0.21	0.48 ^B ±0.09	0.0002
MRT (hr)	23.85 ^A ±3.10	41.95 ^A ±36.83	62.42 ^A ±10.61	128.01 ^B ±23.27	0.0001
Vd (mL/kg)	205.37 ^A ± 39.01	181.70 ^A ± 30.91	100.45 ^B ± 4.97	59.46 ^B ± 5.79	0.0000

^a Letters (A,B,C) within each parameter denote different compartments ($\alpha=0.05$, significant when $p < 0.05$) with Tukey's HSD. Parameters include A and B = y- intercept for the distribution and elimination phase of the curve, respectively; α and β = the absorption and elimination rate constant, respectively; α and β = the absorption and elimination half lives, respectively; AUCINF= Area under the curve measured to infinity observed; Cl = systemic clearance rate; MRT= mean reside time; and Vd = Volume of distribution. See appendix B for equations.

VITA**Jeremy Dane Perkins****Permanent Address**

3734 Sunset

Snyder, TX 79549

Jeremy_D_Perkins@yahoo.com

Education

2004 Master of Science in Veterinary Physiology, Texas A&M University

1999 Bachelor of Science in Biomedical Sciences, Texas A&M University

Professional Experience

2002-2004 Graduate/Research Assistant to Dr. Dawn Boothe, Department of
Veterinary Physiology & Pharmacology, Texas A&M University.

2001-2002 Oncology Technician, North Texas Veterinary Referral Clinic, Animal
Diagnostic Clinic, Dallas, TX.

2000-2001 Surgical Technician, Texas A&M Small Animal Clinic.