PHARMACOKINETIC REPORT

Pharmacokinetics of orally administered single-dose ponazuril in cats

Catherine Burlison[1] | Sherry Cox[2] | Joseph Smith[3] | Jennifer Stokes[1] | Jacqueline C. Whittemore[4] | Becky DeBolt[1]

[1]Department of Small Animal Clinical Sciences, College of Veterinary Medicine, The University of Tennessee, Knoxville, Tennessee, USA

[2]Department of Biomedical and Diagnostic Sciences, College of Veterinary Medicine, The University of Tennessee, Knoxville, Tennessee, USA

[3]Department of Large Animal Clinical Sciences, College of Veterinary Medicine, The University of Tennessee, Knoxville, Tennessee, USA

[4]Animal Emergency and Specialty Center of Knoxville, Knoxville, Tennessee, USA

Correspondence
Becky DeBolt, Department of Small Animal Clinical Sciences, College of Veterinary Medicine, The University of Tennessee, Knoxville, TN, USA.
Email: bdebolt@utk.edu

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Abstract

Cats and kittens in animal shelters and catteries regularly suffer from severe gastrointestinal coccidiosis, which can be fatal, and there are no drugs labeled for feline coccidiosis in the United States. Ponazuril, a triazine-class drug, is increasingly used at a dose of 50 mg/kg/d, orally, for three to five days in shelter environments for coccidiosis. A single oral dose of ponazuril paste 15% (Marquis®; Merial) at 50 mg/kg was administered to six healthy adult cats. Sample analysis was completed via high-performance liquid chromatography. Plasma concentrations peaked at 7.49 ± 2.06 μg/ml at 14.67 ± 7.45 hr post-administration. This study shows that ponazuril achieved a plasma concentration that inhibits growth of similar organisms after a single oral dose in cats. Further studies are necessary to optimize dosing for the treatment of clinical coccidiosis in cats.

KEYWORDS
cats, coccida, coccidiosis, ponazuril, shelter

1 | INTRODUCTION

Intestinal coccidiosis is a common cause of morbidity and mortality in kittens in animal shelters, and its pathogenesis remains poorly understood. (Dubey et al., 2009) Coccidia are obligate intracellular parasites routinely found in the intestinal tracts of mammals. Like many infectious diseases, age and living conditions appear to be the most influential factors in the prevalence of *Cystoisospora felis and Cystoisospora rivolta*, the most common species of coccidia in cats. Neonates and kittens less than three months old are the most susceptible to experimental infections and have the highest rate of natural infection. (Barutzki & Schaper, 2011; Dubey et al., 2009; Shah, 1971) Breeding facilities and shelters, where the cat populations are dense and the environments could be unhygienic, have higher prevalence of coccidia infected animals. (Dubey et al., 2009; Lloyd & Smith, 2001) Studies in shelters in North America have described prevalence rates from 14% to 21%. (Hoggard et al., 2019; Lucio-Forster & Bowman, 2011; Villeneuve et al., 2015; Wyrosdick et al., 2017) Sources of stress such as weaning, shipping, and change of ownership also contribute to development of symptomatic disease. While many cats do not exhibit clinical signs of coccidiosis, some develop mucoid or hemorrhagic diarrhea leading to weight loss

and dehydration. (Dubey et al., 2009; Lloyd & Smith, 2001) Vomiting, anorexia, lethargy, hypoproteinemia, and death or euthanasia occur with severe infections. (O'Brien et al., 2002).

Intestinal coccidiosis is difficult for shelters to manage as individuals most susceptible to clinical disease also are vulnerable to other life-threatening illnesses. The only Food and Drug Administration (FDA)-approved drug for treatment of coccidiosis in a small animal is the coccidiostatic drug sulfadimethoxine, it is administered for five to 21 days for coccidia, which makes it intensive from a time and economic standpoint. Lloyd and O'Brien reported in the early 2000s that toltrazuril reduces coccidia oocyst shedding and lessens diarrhea in kittens. (Lloyd & Smith, 2001; O'Brien et al., 2002) Dosing recommendations have ranged from only one dose, one dose followed by a second dose five days later, to daily doses for three or five days. Unfortunately, toltrazuril is not available in the United States of America (US). Ponazuril, a metabolite of toltrazuril, is available in the United States, which is commonly used even though it can be harder to accurately dose due to the concentration of the commercially available product. A recent study by Litster demonstrated the highest reduction of fecal oocysts when naturally infected shelter cats were dosed with 50 mg/kg of ponazuril for three consecutive days. This is a much higher dosage than recommended for other species. Of the cats, 12.5% had detectable oocysts at days three and four post-treatment. (Litster et al., 2014) It is unknown whether persistent shedding was due to inadequate absorption and, thus, subtherapeutic blood concentrations in some cats. If so, a higher dosage might be more efficacious in treating gastrointestinal coccidiosis. The objective of this study was to obtain pharmacokinetic parameters for a single oral administration of ponazuril (50 mg/kg) in healthy adult cats.

2 | MATERIALS AND METHODS

2.1 | Experimental subjects

Six healthy, female adult, purpose-bred domestic short hair cats from a research colony at the University of Tennessee, Knoxville, were used. Cats were excluded if abnormalities were present on laboratory diagnostics performed two months prior to the study (CBC, serum biochemistry profile, and urinalysis), or if there were any physical examination findings suggestive of systemic or gastrointestinal disease including poor hair coat, low body or muscle condition score (<5/9 or <3/3, respectively), or abnormalities identified on abdominal palpation or thoracic auscultation. The cats weighed 3.58–5.08 kg with a mean of 4.53kg. The study was approved by the Institutional Animal Care and Use Committee at the University of Tennessee, Knoxville (Protocol #2783-0920).

Studies using six animals allow the acquisition of precise preliminary estimates of the average pharmacokinetic parameters. (Riviere, 2011).

Twenty-four hours prior to ponazuril administration, 24G long line sampling catheters (MILA International) were placed after intramuscular sedation with 40 μg/kg dexmedetomidine (Dexdomitor®;

Orion Co.) and 5 mg/kg ketamine (Zetamine®; Vet One). The following day (24 hr later), the cats were administered 50 mg/kg of ponazuril paste 15% (Marquis®; Merial), orally and monitored for complete ingestion. Ponazuril was weighed to the nearest microgram to ensure dose accuracy. Sampling catheters were removed within two days of placement due to decreased blood sampling frequency or lack of patency. Blood was collected at time 0, 15, and 30 minutes, then 1, 2, 4, 8, 12, 24, 48, 72, 96, 120, 144, 168, 192, 216, 240, 264, 288, 312, and 336 hr. These time points were chosen based on clearance times observed in other species. (Dirikolu et al., 2009; Prado et al., 2011; Zou et al., 2014) Most samples consisted of 1ml of blood; no more than 24 ml of blood was taken from one cat over the 2 week period. For samples taken via catheter, a 3 ml sample was withdrawn prior to collection of the sample for testing, which was returned prior to flushing the catheter to limit the total volume of blood removed from each cat. The location of venipuncture for samples taken after catheters were removed alternated on the right and left sides from lateral saphenous, medial saphenous, cephalic, and jugular veins. Plasma was spun at 3,000 *g* for 15 min and frozen at −80°C and assayed within 2 weeks of collection. Cats were monitored for lethargy, restlessness, change in appetite, vomiting, and diarrhea twice daily throughout the study.

2.2 | Extraction method

Ponazuril was extracted from plasma samples using a liquid-liquid extraction method. (Cox et al., 2021) Previously frozen plasma samples were thawed, vortex-mixed, and 100 μl of plasma was transferred to a 13 × 100 mm screw top tube followed by 10 μl of diclazuril (internal standard, 100 μg/ml) and 2 ml chloroform. The tubes were rocked for 15 min and then centrifuged for 20 min at 1000 *g*. The organic layer was transferred to a clean tube and evaporated to dryness with nitrogen gas. Samples were reconstituted in 250 μl of mobile phase and 100 μl was analyzed.

2.3 | Chromatographic conditions and apparatus

The system consisted of a 2695 separations module, a 2487 UV absorbance detector, and a computer equipped with Empower software (Waters). The compounds were separated on a Symmetry RP_{18} (4.6 × 150 mm, 5 μm) column with a Symmetry Shield RP_{18} (3.8 mm × 5 mm × 5μm) guard column. The mobile phase was a mixture of 0.1% formic acid in water and acetonitrile (50:50 v/v). Absorbance was measured at 254 nm with a flow rate of 1.1 ml/min and the column temperature was maintained at 23°C.

2.4 | Calibration

Calibration plasma samples were obtained from six different cats not treated with ponazuril, to verify the possibility of any interfering

matrix compounds near the retention time of ponazuril, and were prepared exactly as study plasma samples. The standard curve was composed of 0.1, 0.25, 0.5, 1, 2.5, 5, 10 and 25 µg/ml concentrations, which were chosen based on expected results of study samples. Standard curves for plasma analysis were prepared by fortifying untreated, pooled plasma with ponazuril to produce a linear concentration range of 0.1 – 25 µm/ml.

2.5 | Pharmacokinetic analysis

Computer software (Phoenix 64 WinNonlin 8.1, Pharsight Corp) was used to calculate pharmacokinetic parameters for ponazuril, including elimination half-life (t½), elimination rate constant (λz), maximum plasma concentration (C_{max}), time to maximum plasma concentration (t_{max}), area under the plasma concentration-time curve from time 0 to last point (AUC_{0-last}), area under the plasma concentration-time curve from time 0 to infinity ($AUC_{0-\infty}$), percent of the $AUC_{0-\infty}$ extrapolated to infinity (AUC_{extrap}), and mean residence time ($MRT_{0-\infty}$) using non-compartmental analysis. Variability in pharmacokinetic parameters was expressed as the standard deviation. In the case of the half-life, the harmonic mean and pseudostandard deviation were used instead.

3 | RESULTS

The average recovery of ponazuril for the quality control (QC) samples (0.3, 7.5, and 15 µg/ml) and the LLOQ was 101% ± 0.5%. This was obtained for the liquid-liquid extraction method from feline plasma using a reversed phase high performance liquid chromatography method. The intra-assay variability for the QC samples ranged from 3.7% to 9% while the accuracy was 100%. The inter-assay variability ranged from 4.9% to 7.6% and the accuracy ranged from 96% to 104%. The lower limit of quantification (LLOQ) was 0.1 µg/ml. The accuracy and precision for the method was considered acceptable based on the FDA 2018 guidelines. (Food and Drug Administration, 2018)

Mean plasma pharmacokinetic parameters and the plasma concentration-time profile are shown in Table 1 and Figure 1. Analysis of plasma samples collected following oral administration of a single dose of ponazuril (50 mg/kg) to six healthy cats confirmed systemic absorption of the drug. Plasma samples obtained at time 0 minutes had no detectable concentrations of ponazuril. Peak plasma concentration of 7.49 ± 2.06 µg/ml occurred at 14.67 ± 7.45 hr. Plasma concentrations declined to 1.57 ± 0.92 µg/ml with an average elimination half-life of ~136 hr. Plasma concentrations were still detectable at 336 hr after ponazuril administration. No side effects were observed.

4 | DISCUSSION

Ponazuril, (1-methyl-3-[3-methyl-4-(4-trifluoromethanesulfon yl-phenoxy)-phenyl]-[1,3,5]-triaziane-2,4,6-trione), is a triazine

TABLE 1 Pharmacokinetic parameters (mean ± SD) in cats following oral administration of 50 mg/kg ponazuril (n = 6)

Pharmacokinetic parameter	Ponazuril mean ± SD
Elimination half-life t½ (h)*	136 ± 48
Elimination rate constant λz (1/h)	0.006 ± 0.002
t_{max} (h)	14.67 ± 7.45
C_{max} (µg/ml)	7.49 ± 2.06
AUC_{0-last} (h·µg/ml)	1302 ± 490
$AUC_{0-\infty}$ (h·µg/ml)	1649 ± 733
AUC_{extrap} (%)	18.6 ± 9.9
$MRT_{0-\infty}$ (h)	208 ± 67

Note: Elimination half-life (t½), elimination rate constant (λz), maximum plasma concentration (C_{max}), time to maximum plasma concentration (t_{max}), area under the plasma concentration-time curve from time 0 to last point (AUC_{0-last}), area under the plasma concentration-time curve from time 0 to infinity ($AUC_{0-\infty}$), percent of the $AUC_{0-\infty}$ extrapolated to infinity (AUC_{extrap}), mean residence time ($MRT_{0-\infty}$).

derivative coccidiocidal drug that has been studied in numerous species including swine, llamas, cattle, goats, horses, and dogs. (Charles et al., 2007; Dirikolu et al., 2009; Furr & Kennedy, 2000; Love et al., 2015; Prado et al., 2011; Zou et al., 2014) It is available in the United States as Marquis® Paste, and is labeled to treat *Sarcocystis neurona* infection in horses. Ponazuril is an effective anti-coccidial medication in chickens, calves, and buffalo. (Epe et al., 2005; Ghanem et al., 2008; Laczay et al., 1995) Previous pharmacokinetic studies have determined absorption of single-dose oral administration of ponazuril in cattle, goats, horses, llamas, and swine. (Dirikolu et al., 2009; Furr & Kennedy, 2000; Love et al., 2015; Prado et al., 2011; Zou et al., 2014) Pharmacokinetic parameters vary dramatically by species. Both Dirikolu, et al. and Prado, et al. proposed that the variability of half-life among species may reflect a difference in the rate of oral absorption rather than elimination rate. (Dirikolu et al., 2009; Prado et al., 2011) The rate of absorption can be affected by binding to food in the gastrointestinal tract. The earliest detection of plasma ponazuril above the LLOQ occurred at 30 min in 50% of the subjects, with detection in all cats at 1 hr. The t_{max} was 14.67 ± 7.45 hr, indicating absorption rate was much faster in cats than llamas, goats, swine, and cattle; (Dirikolu et al., 2009; Love et al., 2015; Prado et al., 2011; Zou et al., 2014) however, the determination of t_{max} can be dependent on sampling schedule and the clinical significance of this difference is unknown. The C_{max} ranged from 5.4 to 10.4 µg/ml with a mean of 7.49 ± 2.06 µg/ml, which is higher than cattle or swine but lower than llamas, horses, and goats. (Dirikolu et al., 2009; Furr & Kennedy, 2000; Love et al., 2015; Prado et al., 2011; Zou et al., 2014) It should be noted for the C_{max} comparisons that these other studies used lower dosages than the cats in this study, with llamas receiving 20 mg/kg, goats and horses receiving 10 mg/kg, and cattle and pigs receiving 5 mg/kg. The mean t½ of ponazuril in cats was similar to that of goats, llamas, and swine, whereas it was greater than two-fold longer than in cattle or horses. (Dirikolu et al., 2009;
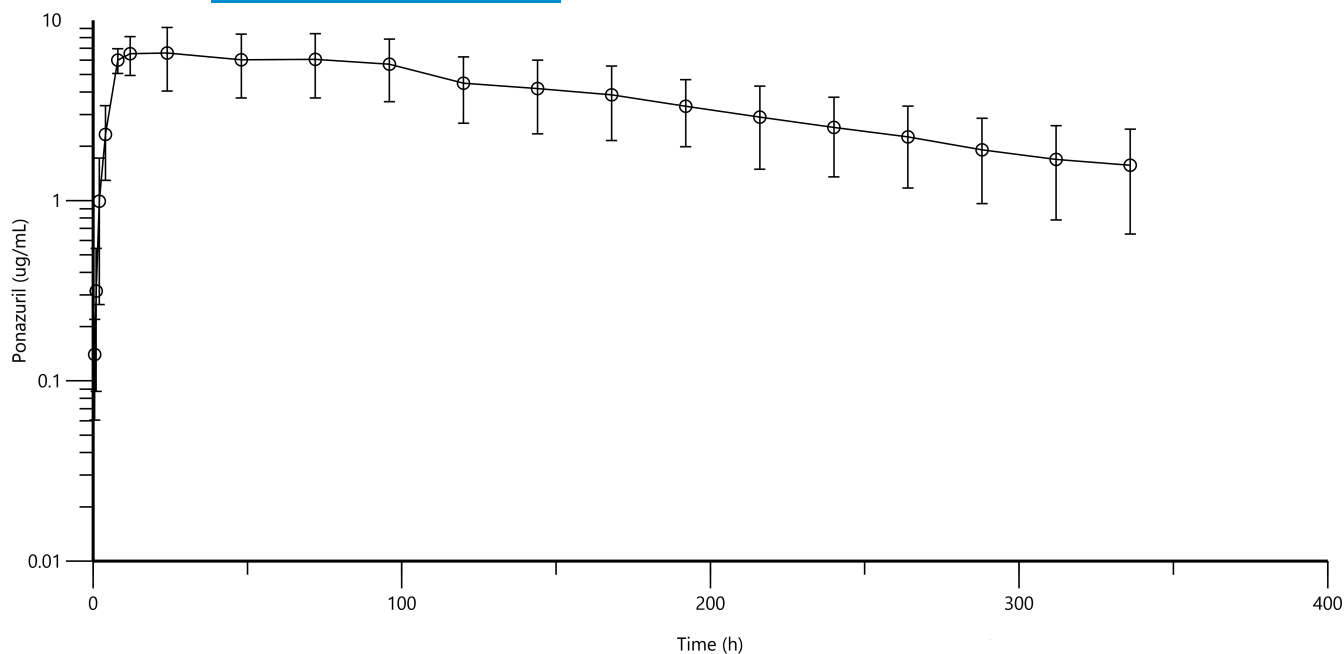
FIGURE 1 Mean plasma concentration-time curve for ponazuril following oral administration of a single dose (50 mg/kg) to 6 healthy cats. Values reported are mean ± SD

Furr & Kennedy, 2000; Love et al., 2015; Prado et al., 2011; Zou et al., 2014).

Limitations of this study include the inability to study bioavailability and the sampling timeframe. An injectable formulation of ponazuril is not available; therefore, the oral bioavailability of ponazuril in cats cannot be determined from our study; however, future studies could consider preparing ponazuril in a suitable vehicle for intravenous administration. Sample collection was stopped 14 days after ponazuril administration, at which point ponazuril was still measurable. When the last measured concentrations are above the assay lower limit of quantification, parameters such as half-life and mean residence time can be artifactually elevated, (Smith et al., 2018) as the ability to detect lower concentrations for a longer period of time will extend elimination half-life and mean residence time in a mathematically correct manner, but not in a clinically relevant one. Future studies investigating the pharmacokinetics of ponazuril in cats should employ a longer sampling regimen to more completely capture elimination kinetics. Although we did estimate the apparent elimination half-life, a large portion of the AUC was extrapolated. This suggests that samples should have been collected for an extended timeframe to more accurately estimate the elimination half-life.

With no in vitro studies exploring the sensitivity of C. felis or C. rivolta to ponazuril, it is not possible to determine if the anecdotal dosage needed to decrease oocyst shedding in cats (50 mg/kg) is due to properties of feline metabolism of the drug or decreased sensitivity of Cystoisospora spp to the drug itself. With this dosage, a plasma concentration was reached that has been shown to inhibit growth in similar organisms, in vitro, and is effective against Apicomplexans and used therapeutically in other species. (Alnassan et al., 2013; Furr et al., 2001; Gibbons et al., 2016; Joachim & Mundt, 2011; Jonsson et al., 2011; Kritzner et al., 2002; Mitchell et al., 2005; Redrobe et al., 2010) Robust pharmacodynamic studies, in which repeated dosing pharmacokinetics are paired with assessment of efficacy in the shelter environment, must be completed to validate the clinical dosage and efficacy of ponazuril against intestinal coccidiosis in cats.

CONFLICT OF INTEREST

The authors disclose no conflict of interest.

AUTHOR CONTRIBUTION

CDB contributed to sample collection, data analysis, and manuscript writing. SC led analytical method development, sample and data analysis, and contributed to study design and manuscript preparation. JSS was involved in sample collection, data analysis, and manuscript preparation. JES contributed to overall study execution and manuscript preparation. JCW contributed to overall study execution and manuscript preparation. BKD was involved in study design, sample collection, data analysis, and manuscript preparation. All authors have read and approved the final manuscript.

ETHICAL APPROVAL

DATA AVAILABILITY STATEMENT

ORCID

Catherine Burlison https://orcid.org/0000-0001-9718-2573
Sherry Cox https://orcid.org/0000-0002-5184-900X
Joseph Smith https://orcid.org/0000-0002-4288-2262
Jennifer Stokes https://orcid.org/0000-0002-6728-2116
Jacqueline C. Whittemore https://orcid.org/0000-0003-2624-2262
Becky DeBolt https://orcid.org/0000-0003-0382-6556

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