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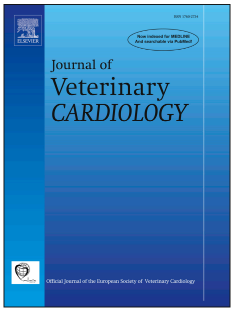

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The effect of excipients on the oral absorption of pimobendan in healthy dogs

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The authors declare no off-label use of antimicrobials. Generative AI-assisted technologies were not used in the generation of this manuscript.

All activities were approved by the Institutional Animal Care and Use Committee of the University of Georgia (AUP: A2022 06-014-Y1-A0).

Abstract

Introduction/Objectives. Compounded pimobendan is prescribed without knowledge of the effect of excipients. The objective of this study was to test the effect of certain excipients on oral absorption in dogs by describing the single-dose pharmacokinetics of pimobendan and its active metabolite (o-desmethyl-pimobendan [ODMP]) following oral administration of two experimental pimobendan formulations, and to compare these pharmacokinetic estimates to those of an FDA-approved product (Vetmedin®).

Animals. Seven healthy adult purpose-bred dogs

Methods. Dogs were administered 2.5 mg of pimobendan as a single oral dose of Vetmedin®, or of an experimental capsule with or without citrate as an excipient, in a randomized, prospective cross-over study with a two-week washout period between treatments. Blood samples were obtained at pre-determined timepoints before and for 24 hours post-dose for quantification of pimobendan and ODMP using ultra-high-pressure liquid chromatography with mass spectrometry. Compartmental analysis was used to obtain pharmacokinetic parameters. Data are presented as geometric mean (CV%).

Results. Peak plasma pimobendan concentrations (C_{MAX}) were 8.82 ng/mL (53%), 5.85 ng/mL(76%), and 8.11 ng/mL (70%), and areas under the concentration-time curve (AUC) were 19.0 hr*ng/mL (55%), 13.6 hr*ng/mL (68%) and 19.7 hr*ng/mL (40%) for Vetmedin®, citrate-containing capsules and citrate-free capsules, respectively. Experimental formulations had high relative absorption ($AUC_{(test\ drug)}/AUC_{(Vetmedin®)}$, 72% [61%] and 103% [71%] for citrate-containing and citrate free-capsules, respectively).

There was moderate-to-high inter-individual pharmacokinetic variability for all formulations. No differences in C_{MAX} or AUC were found among treatments.

Study limitations. Small sample size

Conclusions. This study did not identify differences in relative bioavailability among the tested formulations; however, bioequivalence should not be assumed. Substantial inter-individual pharmacokinetic variability was noted.

Keywords: relative bioavailability, drug concentration

Abbreviations Table:

AUC	area under the plasma drug concentration-time curve
C_{MAX}	peak plasma drug concentration
CV%	coefficient of variation
FDA	Food and Drug Administration
K1	absorption rate
K10	elimination rate
ODMP	o-desmethyl-pimobendan
T_{MAX}	time to peak plasma drug concentration
USP	United States Pharmacopoeia

Introduction/Objectives

Pimobendan is a benzimidazole-pyridazinone derivative inodilator that is widely prescribed for pre-clinical canine valvular heart disease and cardiomyopathy, and has become a mainstay of treatment for dogs with congestive heart failure, supported by the conclusions of several prospective, randomized, controlled clinical trials [1-5]. A recent survey of electronic health records from over one million dogs in the United Kingdom identified pimobendan as the second-most prescribed cardiovascular agent behind furosemide, recommended in 52% of all consultations in which a cardiovascular medication was ultimately prescribed [6].

In the United States, two Food and Drug Administration (FDA)-approved oral pimobendan formulations[e,f] are available for prescription in dogs. In addition, compounded pimobendan formulations are produced by various compounding pharmacies in the United States. However, pimobendan is *not* listed among the bulk substances under which the FDA does not intend to take enforcement action for violations of the Food, Drug, and Cosmetic Act with respect to the compounding of animal drugs from bulk drug substances [7]. Therefore, it is a violation of the Federal Food, Drug, and Cosmetic Act to compound pimobendan for animals from bulk drug substances. Regardless of this fact, we have observed that this is common practice among compounding pharmacies.

Pimobendan is poorly soluble in aqueous media and has pH-dependent solubility, which necessitates dissolution in an acid environment to ensure constant, satisfactory absorption from the canine gastrointestinal tract [8]. In people and dogs, when pimobendan is not mixed with an acid excipient, low plasma drug concentrations and high between-subject variability are observed. This variability can be partially overcome by including a citric acid excipient in the pimobendan oral formulation, which has been reported to increase the solubility by a factor of 100 when compared to artificial gastric juice [8].

Vetmedin®, a United States FDA-approved formulation of pimobendan, contains citric acid as an excipient. However, formulations produced by two major United States compounding pharmacies did not at the time of personal communication (March 2022), raising concern for potentially unreliable solubility and bioavailability of these products. Compounding pharmacies in the United States have not provided customers bioequivalence data for their products compared to the proprietary formulation of Vetmedin®. This raises questions about the performance of these products in dogs with heart disease.

The objective of this study was to test the effect of certain excipients on the oral absorption of pimobendan in healthy dogs. To this aim, we sought to describe and compare plasma pharmacokinetics of pimobendan and its active metabolite, o-desmethyl-pimobendan (ODMP), following administration of a single oral dose of one of two experimental pimobendan capsules – one with citric acid as an excipient and one without – or Vetmedin® to healthy dogs. We hypothesized that the oral absorption of pimobendan would be significantly lower and inconsistent if the citric acid excipient was not included, and, importantly, that Vetmedin® would be significantly better absorbed than either of the experimental products, indicating that excipients in addition to citric acid are important for product performance. This study was not designed to evaluate bioequivalence.

Animals, Materials and Methods

Study design. This was a prospective, randomized, cross-over, single-dose experimental study conducted from October to November 2022. All activities were approved by the Institutional Animal Care and Use Committee of the University of Georgia (AUP: A2022 06-014-Y1-A0).

Animals. Seven healthy, adult, sexually intact, female, purpose-bred beagle dogs were used. Prior to the beginning of the study, each dog's general and cardiac health was confirmed by normal findings of physical examination, complete blood count, serum biochemical profile, and urinalysis. Dogs were pair-housed except during blood sampling periods when they were housed individually. Dogs had free access to water and were fed a commercially available adult canine ration twice daily. Ambient temperature was maintained between 20° and 22°C, inclusive, and dogs were exposed to a 12-hour light-to-dark cycle.

Study drug administration. Dogs were administered 2.5 mg pimobendan orally as a single dose of Vetmedin®[e], or as one of two experimental formulations of pimobendan, one with and one without citrate as an excipient. This dose was based on the recommendations of the FDA-approved product's manufacturer and the starting dose recommended by veterinary cardiologists for the treatment of heart disease and failure in dogs [2]. The dose administered to dogs was not based on body weight to avoid dose variability that might be introduced by splitting tablets; however, dogs of similar body weight were selected to provide a uniform mg/kg dose. Each dog received all three treatments with a two-week washout period between treatments. The washout period was chosen to be sufficiently long for complete elimination of the previous dose, as well as to allow for replenishment of blood volume after collecting samples. On sampling days, food was withheld for 12 hours prior to drug administration and was offered approximately four hours after dosing. Water was available *ad libitum* during the sampling period.

Initially, six dogs were randomized to one of six treatment sequences using a balanced Latin Square design [9] generated for three conditions using an online tool[g] such that each treatment preceded the other exactly twice. A random number generator[h] was used to assign each of these six dogs a unique sequence number. Unexpectedly, a seventh dog was obtained prior to the start of the study; the treatment sequence for this dog was assigned by choosing one of the six existing treatment sequences at random.

Pimobendan preparation. Experimental pimobendan formulations were prepared on-site at the University of Georgia by a licensed pharmacist (DLE) according to United States Pharmacopoeia (USP) standards for non-sterile compounding[i]. The formulations were administered within 30 days of production to comply with the beyond-use-date of the USP standard. In-house production permitted quality control and elimination of variables that might be introduced by uncertainties relative to shelf time, shipping conditions (e.g., extremes of temperature) and inter-batch variability.

Both experimental pimobendan formulations were hard gelatin capsules containing 2.5 mg of the active pharmaceutical ingredient and several excipients, one formulation with (citrate-containing) and one formulation without (citrate-free) citric acid (**Table 1**). Capsules were prepared in batches of 10 by reducing the particle size of each powdered ingredient separately using a glass mortar and pestle; weighing the appropriate amount of each triturated ingredient using an electronic balance with a readability of .0001g; combining all ingredients in a glass mortar using geometric dilution and trituration until a homogenous powdered mixture was achieved; sieving the powdered mix; and hand-packing the finished mixture (268 mg per capsule) into size two gelatin capsules[j]. Citrate-free capsules were prepared identically, except for the omission of citric acid and a finished mixture weight of 59 mg per capsule. Capsules were weighed for accuracy, locked, polished, and stored with a desiccant at room temperature until use.

The formula for experimental citrate-containing capsules was taken directly from “example four” of the United States Patent for oral pharmaceutical forms of pimobendan [8]. This formula was chosen for the present study for several reasons. First, of the formulas for which information is provided in that patent, rate of capsule dissolution (measured using USP methods at a pH of 5.5) is high: 96.5% and 99.1% dissolved after 15 and 30 minutes, respectively [8]. A compressed tablet represents the formulation most frequently prescribed by veterinary cardiologist in the United States according to a recent unpublished survey [10]; however, tablets are labor-intensive to produce on a small scale. Instead, given that the capsule formulation used here was proven bioequivalent to a compressed citric acid-containing tablet formulation in 11 human test subjects, [8] the former was selected for study.

Sample collection and processing. On the day prior to treatment and scheduled blood sampling, an indwelling, single-lumen, 20 G x 12 cm jugular venous sampling catheter[k] was placed percutaneously in each dog. Dogs were sedated with intravenously administered butorphanol (0.3 mg/kg) and dexmedetomidine (5 mcg/kg) to facilitate placement, and the effects of sedation were reversed with intramuscularly administered atipamezole (50 mcg/kg) after successful placement. Catheters were rinsed with heparin saline solution (10 IU/mL). Body weight was measured and recorded on the day prior to sampling.

On treatment days, blood was sampled for the measurement of plasma pimobendan and ODMP concentrations immediately prior to (time 0), and at 0.25, 0.5, 1, 1.5, 2, 3, 4, 8, 12, and 24 hours following oral pimobendan administration. Prior to collection of the first (time 0) blood sample, the heparin saline solution was withdrawn from the catheter lumen along with approximately 1 mL of blood, and both were discarded. The catheter was then rinsed with heparinized saline solution (100 IU/mL). At each sampling time point, 2 mL of blood was withdrawn from the jugular catheter and retained. Then, an additional 2 mL of blood was collected and transferred to a collection tube[l] containing lithium heparin and placed immediately on ice. The retained blood sample, followed by 2 mL of normal saline, were returned through the catheter, and the catheter was rinsed with heparinized saline solution. Blood samples were centrifuged at 2-4℃ and 2000 x g for 10 minutes within 20 minutes of collection. Plasma was harvested from the sample and stored at -80°C.

Plasma pimobendan and o-desmethyl-pimobendan measurement. Plasma samples were shipped overnight on dry ice and stored at -80ºC until analysis. Pimobendan and ODMP concentrations were quantified using ultra-high performance liquid chromatography with tandem mass spectrometry (LC-MS/MS) at the Auburn University Specialized Pharmaceutical and Experimental Center for Translational Research and Analysis, adapted from a previously described method [11].

Reference standards for pimobendan, ODMP, and an internal standard, $^{13}C,^{2}H_3$-pimobendan, were obtained[m]. Analyses were performed using an ultra-high-performance liquid chromatography (UHPLC) system[n] coupled to a mass spectrometer[o] (MS/MS). Samples were introduced into the LC-MS/MS system using electrospray ionization in positive-ion mode for detection. Data processing was performed using a commercial software package[p]. Stock solutions of pimobendan, ODMP, and $^{13}C,^{2}H_3$-pimobendan were prepared at 1 mg/mL and 100 µg/mL. The 1 mg/mL stock solutions were prepared and diluted in methanol and stored at -20ºC. Calibration solutions were prepared in blank dog plasma at concentrations ranging from 150 ng/mL to 0.037 ng/mL. Quality control samples were prepared in triplicate from separately prepared stock solutions at concentrations of 0.293, 2.344, and 37.5 ng/mL.

Calibration, quality control, and test samples (200 µL each) were deproteinated by the addition of 800 µL acetonitrile[q] followed by vortexing for 20 sec and centrifugation (13,000 x g, 4ºC). Prior to deproteinization, 10 µL of internal standard was added to all standards, quality control, and test samples to achieve a concentration of 30 ng/mL. Following centrifugation, 600 µL of supernatant was transferred to an HPLC vial, and 200 µL of H_2O were added to each to aid in separation and peak shape.

A 2 µL sample was injected into UHPLC-MS/MS system via autosampler, and separation was performed on a column[r] (2.1x50 mm, 1.8 µm) maintained at 40ºC. A gradient elution was performed according to the following timing: 95% to 60% mobile phase A (H_2O + 0.1% [V/V] formic acid[s]) and 5% to 40% mobile phase B (acetonitrile + 0.1% [V/V] formic acid) from 0 to 0.7 min, at a flow rate of 0.6 mL/min. Retention times of ODMP, pimobendan, and $^{13}C,^{2}H_3$-pimobendan were 1.047, 1.166, and 1.164 min, respectively, with a total run time of 2.2 min. During assay development, blank samples were injected throughout the analysis of standard and incurred samples to ensure chromatographic response returned to baseline prior to subsequent injections. During the analysis of all samples, blank samples were also used after standards and in conjunction with quality control samples to monitor the performance of the assay.

Quantitation was performed using multiple reaction monitoring mode to monitor parent-to-product-ion (m/z) transitions for pimobendan, ODMP, and $^{13}C,^{2}H_3$-pimobendan. Both a quantifying and qualifying ion were selected for each analyte. The standard curve was linear with concentrations within 10% of their nominal values, and QC samples were within 10% of their nominal values with <15% coefficient of variation. No evidence of carry-over, alterations in baseline, or changes in pump pressure were observed during the analysis.

Pharmacokinetic analyses. Pharmacokinetic analyses and modeling of sample concentrations were performed using a commercially available software program[t]. Plasma pimobendan and ODMP concentrations were indexed for dose (mg/kg) by multiplying each by a scalar of dose ratio prior to analysis. Compartmental methods were used to analyze plasma pimobendan and ODMP concentration data. Values less than the assay lower limit of quantification were not included in the pharmacokinetic analysis.

In addition to the primary pharmacokinetic parameters, for the parent drug (pimobendan) oral absorption and peak plasma concentration (C_{MAX}) relative to those of Vetmedin® were calculated for each dog and experimental treatment as: Relative absorption (%) = $AUC_{(experimental\ formulation)}$ / $AUC_{(Vetmedin®)}$ ×100 and Relative C_{MAX} (%) =

$C_{MAX(experimental\ formulation)}$ / $C_{MAX\ (Vetmedin®)}$ ×100, respectively, where AUC is area under the plasma drug concentration-time curve. The metabolite-to-parent AUC ratio (%) was also estimated for each dog and experimental formulation as: $AUC_{(ODMP)}$ / $AUC_{(pimobendan)}$ ×100.

Statistical analyses. Statistical analyses were performed using two commercially available software packages[t,u]. Descriptive statistics are presented as arithmetic mean $\pm$ SD or median (range) for sample data and geometric mean (geometric CV% [appropriate for log-transformed variables]) for pharmacokinetic data, unless otherwise noted. A linear mixed model was used to test for effects of drug formulation on each of C_{MAX}, time to C_{MAX} (T_{MAX}), AUC, and elimination rate (K10). The model had a fixed factor for drug formulation and a random intercept for dog. Model assumptions (normality and homoscedasticity of model residuals) were evaluated via inspection of QQ-plots, histograms, and conditional residual plots. Multiple comparisons were adjusted for using Tukey's test. Satterthwaite degrees of freedom method and restricted maximum likelihood estimation were used. A significance threshold of 0.05 was used.

The sponsor of Vetmedin® has reported mean peak plasma pimobendan concentration (C_{MAX}) of 3.09 ng/mL (standard deviation, 0.76 ng/mL) following a single oral dose of 0.25 mg Vetmedin®/kg [12]. Based on this, it was determined that information from 7 dogs would have 77% power to detect a 35% difference in C_{MAX}, assuming type 1error rate (alpha) = 0.05.

Results

Dogs had a median (range) age of 42 months (22 – 45 months) and mean ± SD body weight of 6.89 kg ± 0.45 kg. The three pimobendan formulations were well tolerated by all dogs, with each dog receiving the entire drug dose for all three treatments. Blood samples of adequate volume were collected from all dogs at all time points. Median (range) doses of pimobendan administered were 0.362 mg/kg (0.343 – 0.424 mg/kg), 0.362 mg/kg (0.343 – 0.417 mg/kg) and 0.357 mg/kg (0.347 – 0.417 mg/kg) for Vetmedin®, citrate-containing capsules, and citrate-free capsules, respectively.

Plasma concentrations of pimobendan and ODMP following the administration of each of the three pimobendan formulations are depicted in **Figure 1**. Concentration-time plots of pimobendan and ODMP concentrations for individual dogs are presented in **Supplemental Figures 1 and 2**, respectively.

The compartmental model providing the best fit for plasma concentrations of both pimobendan and ODMP was a one-compartment model with first-order input and elimination. Summary pharmacokinetic parameters are presented in **Table 2**.

All formulations were relatively rapidly absorbed and converted to the active metabolite, with geometric mean $T_{MAX} < 1.0$ h and < 2.0 h for the parent compound and ODMP, respectively, for all treatments. There were no statistically significant differences between formulations for pimobendan C_{MAX} ($p = 0.49$), AUC ($p = 0.48$), K10 ($p = 0.70$) or T_{MAX} ($p = 0.75$); for all pair-wise comparisons, adjusted P-values were ≥ 0.49. Both experimental formulations had high mean absorption relative to Vetmedin® with the citrate-free formulation having greatest absorption of the three formulations, on average. (**Table 1**)

For all three tested formulations, there was a moderate to high degree of inter-individual variability in all pharmacokinetic parameters, as illustrated by the coefficients of variation (**Table 2**), and by **Supplemental Figures 1 and 2.**

For all tested formulations, AUC and plasma concentrations of ODMP were greater and longer sustained compared to the parent drug. Geometric mean metabolite-to-parent AUC ratios were similar for all formulations, with $AUC_{ODMP}/AUC_{pimobendan}$ > 100% in all but one dog treated with the citrate-free formulation (for which $AUC_{ODMP}/AUC_{pimobendan}$ was 98%).

Discussion

The present study describes the pharmacokinetics of two experimental oral pimobendan formulations – one containing citric acid as an excipient and one not – and compared these characteristics to those of the only FDA-approved formulation available at the time of study (Vetmedin®), in healthy dogs. Our results showed a moderate to high between-subject pharmacokinetic variability of orally administered pimobendan in healthy dogs across the examined formulations. Significant differences were not observed for absorption among the formulations evaluated. We acknowledge the possibility of a Type II statistical error because of the high variation among subjects.

It constitutes a violation of the Federal Food, Drug, and Cosmetic Act to compound pimobendan for animals from bulk drug substances [7]. However, these formulations are frequently produced in the United States. In a 2020 survey of cardiology diplomates of the American College of Veterinary Internal Medicine, 124 (92%) of 135 respondents reported having prescribed pimobendan through a compounding pharmacy in the United States [10]. Pimobendan is poorly soluble in aqueous media and has highly-pH-dependent solubility, leading to considerable inter- and intra-individual variability in absorption [8, 13]. According to a United States patent, the drug should be simultaneously administered as an intimate dry mixture with an acid (specifically, citric acid) at a ratio of 1:10 to 1:20 (pimobendan: citric acid) and processed with excipients to form a powder, pellets or granules for oral administration [8]. In addition to acting as an acid and solubilizing agent, citric acid also serves as a stabilizer for the active substance solution. Because citric acid is hygroscopic, the addition of disintegrants is recommended to ensure that the preparation disintegrates rapidly on contact with the gastric juice, allowing for the formation of acidic microspheres for dissolution (and therefore, absorption) of pimobendan [8]. Subsequent patented approaches sought to overcome the pH-dependency of pimobendan dissolution by preparing pimobendan in a novel crystalline form [14]. or coating particulate pimobendan with an appropriate carrier matrix (e.g., lipid of appropriate size and melting point) to produce soluble microspheres [13]. Collectively, this information suggests that whether a formulation contains citric acid as an excipient, is administered in a crystalline form, or undergoes coating and microencapsulation, might have implications for solubility (and therefore bioavailability) of the product over a range of gastric pH values.

Both experimental pimobendan formulations evaluated here were relatively well-absorbed when compared to Vetmedin®. We did not observe differences in absorption among the formulations evaluated, which we suspect might be due, at least in part, to the high degree of variability among individuals. Similarly, the AUC ratios of ODMP-to-pimobendan were consistent among the three formulations, suggesting that formulation does not affect conversion of the parent drug to metabolite.

Many of our findings are similar those previously reported in dogs administered pimobendan, including rapid absorption and first-order elimination following oral dosing. Mean pimobendan C_{MAX} and AUC values in the present study are comparable to those (7.3 ± 2.7 ng/mL and 22.5 ±10.4 ng*hr/mL, respectively) reported in a prior pharmacokinetic study in which dogs were administered a comparable oral dose (i.e., 0.3 mg/kg) of pimobendan as an oil-based suspension [15]. The sponsor of Vetmedin® [12] and other investigations [16-18] of pimobendan administered to healthy dogs report both lower and higher values compared to our results. Without additional study, the reason for differences between the findings of other studies and ours is undetermined.

Awareness of inter-individual pharmacokinetic variability for a given drug can provide guidance for the safer and more individualized use of drugs, limiting both adverse events and treatment ineffectiveness. Pharmacokinetic variability is expressed by the coefficient of variation (CV) for pharmacokinetic parameters of interest, with CV > 40% proposed to represent high, and CV < 40% proposed to represent low or moderate, variability [19]. Except for one parameter of one formulation (Vetmedin® T_{MAX}, CV 20.76%), the CV for C_{MAX}, T_{MAX} and AUC of the parent compound were all consistent with high inter-individual pharmacokinetic variability across these three formulations. (Table 2) Relative bioavailability with Vetmedin® used as the reference formulation showed high inter-individual variability for both experimental formulations (range of CV, 58 – 95%). Inter-individual pharmacokinetic variability has been previously reported for various formulations of pimobendan; information from the small number of published studies from which CV could be calculated is summarized in **Table 3**. In addition, a recent population pharmacokinetic study of 57 client-owned dogs with naturally occurring myxomatous mitral valve disease, all administered the proprietary formulation of Vetmedin®, documented high between-subject pharmacokinetic variability that could not be explained by disease stage, body weight, dose, or other covariates [20]. Another study showed no difference in response between dogs administered standard-dose or high-dose pimobendan [21], which may reflect within-subject variability in oral absorption rather than a ceiling of therapeutic response. Taken together, we believe the results of our study and others [12, 16, 17, 20] show a high degree of inherent variability of oral pimobendan absorption in dogs. Veterinarians might consider this variability when faced with unexpected response to the drug in clinical practice.

Our hypothesis that pimobendan oral absorption is greater after administration of the proprietary Vetmedin® compared to a citrate-free formulation was true for only three (43%) of the seven dogs evaluated, with the rank order of absorption for the three formulations as follows in the remaining dogs: citrate-free capsule > Vetmedin® > citrate-containing capsule (n = 3), and citrate-free capsule > citrate-containing capsule > Vetmedin® (n = 1; data not presented). In the most extreme cases, relative to values for Vetmedin®, pimobendan C_{MAX} and AUC for the experimental formulations ranged from 6.0-fold lower to 3.7-fold higher and from 2.7-fold lower to 2.3-fold higher, respectively, illustrating the range of variability and rank across formulations within an individual dog.

The high inter-individual pharmacokinetic variability observed for the reference formulation suggests that product dissolution might not fully explain variability in absorption of pimobendan in dogs. Indeed, oral drug absorption is complex and involves more than product dissolution [22-24]. Absorption can be highly variable both between and within subjects, with variability of factors such as gastrointestinal pH and gastrointestinal transit time frequently implicated [22]. Physiochemical characteristics alone do not predict oral absorption of drugs in dogs [24], in which numerous factors can influence drug oral absorption, including breed, diet, body size, host microbiota, and the presence or severity of gastrointestinal pathology [25, 26]. To minimize these influences, dogs in the present study were all female dogs of the same breed with similar body weight, age, and health status. To minimize differences attributed to diet, they were all were fed the same diet before and during the study. To reduce the potential influence of feeding on stomach emptying and absorption [27], feeding schedules were standardized and drug was given after a 12-hour fast, followed by offering food at a consistent time after administration of oral pimobendan.

We were unable to identify a compounding pharmacy that was willing to provide formulas for their compounded pimobendan products. Therefore, it is not possible to know whether the experimental capsules used here accurately represent the products dispensed by compounding pharmacies. In-house production permitted quality control and elimination of variables that might be introduced by uncertainties relative to shelf time, shipping conditions (e.g., extremes of temperature and humidity) and inter-batch variability, and ensured that USP standards for non-sterile compounding[i] were followed.

The authors of the present study do not advocate the compounding of pimobendan from bulk drug substance in any form.

Limitations

Given the small sample size and high inter-individual variability, the likelihood of type II error must be considered. In addition, we were not able to control or account for potential differences in gastric pH between treatment periods; therefore, it is not possible to know whether and to what extent variability in gastric pH might have impacted our results. Finally, we did not account for treatment sequence effect and the possibility of carry-over effects independent of drug elimination half-life.

Conclusions

The results of the present study suggest the potential for substantial within-subject and between-subject pharmacokinetic variability from oral administration of pimobendan to healthy dogs. A high degree of variability was documented across the three tested formulations. The experimental formulations evaluated here were relatively well-absorbed when compared to the FDA-approved formulation, and we were unable to detect a significant difference in various pharmacokinetic parameters with our sample size among the three tested formulations. Critically, this study was not designed to test bioequivalence; therefore, it should not be inferred based on our results.

Footnotes

[e] Vetmedin®, Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO, USA

[f] Pimomedin™, Cronus Pharma Specialties India Private Ltd.

[g] https://hci-studies.org/balanced-latin-square/

[h] random.org

[i] USP General Chapter <795>, www.USP.org

[j] Professional Compounding Center of America, Houston, TX, USA

[k] Arrow International, Inc.; Teleflex Incorporated, Reading, PA, USA

[l] BD Vacutainer; Fisher Scientific, Pittsburg, PA, USA

[m] Alsachim; Illkirch Graffenstaden, Bas-Rhin, France

[n] 1290 Infinity II LC; Agilent Technologies, Inc., Santa Clara, CA, USA

[o] 6460 triple quadrupole; Agilent Technologies, Inc., Santa Clara, CA, USA

[p] MassHunter Qualitative Analysis B.06.00; Agilent, Santa Clara, CA, USA

[q] VWR International, Radnor, PA

[r] Zorbax SB C18; Agilent Technologies, Inc., Santa Clara, CA, USA

[s] Sigma-Aldrich; Merck KGaA, Darmastadt, Germany

[t] Phoenix® WinNonlin®, version 8.4, Certara, Inc., Princeton, NJ, USA

[u] SAS 9.4, Cary, NC, USA

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Figure Legends.

Figure 1. Concentration-time plots of plasma pimobendan (panel A) and o-desmethyl-pimobendan (ODMP; panel B) concentrations in healthy dogs (n = 7) administered 2.5 mg pimobendan as a single oral dose of Vetmedin® (black squares), a citrate-free experimental pimobendan capsule (white circles), or a citrate-containing experimental pimobendan capsule (red triangles). Symbols and error bars depict group arithmetic mean and SD, respectively. In all dogs, plasma drug concentrations < LOQ were measured in samples taken 12 and 24 hours after administration for pimobendan and 24 hours after administration for ODMP; therefore, data from the hour 24 timepoint are not shown.

Supplemental Figure 1. Plasma pimobendan concentrations in healthy dogs (n = 7; Panels A – G) administered 2.5 mg pimobendan as a single oral dose of Vetmedin® (black squares), a citrate-free experimental pimobendan capsule (white circles), or a citrate-containing experimental pimobendan capsule (red triangles). In all dogs, plasma pimobendan concentrations less than the assay limit of quantification were measured in samples taken 12 and 24 hours after administration; therefore, data from these timepoints are not shown.

Supplemental Figure 2. Plasma O-desmethylpimobendan (ODMP) concentrations in healthy dogs (n = 7; Panels A – G) administered 2.5 mg pimobendan as a single oral dose of Vetmedin® (black squares), a citrate-free experimental pimobendan capsule (white circles), or a citrate-containing experimental pimobendan capsule (red triangles).

In all dogs, plasma pimobendan concentrations less than the assay limit of quantification were measured in samples taken 24 hours after administration; therefore, data from this timepoint are not shown.

Table 1. Composition of experimental citrate-containing pimobendan capsules administered to seven healthy dogs in the present study. The composition of citrate-free capsules was identical except for the omission of citric acid and a final capsule weight of 59 mg. USP, United States Pharmacopoeia; NF, National formulary grade.

Ingredient	Amount per capsule (mg)	Description	Manufacturer
Pimobendan, USP reference standard	2.5	Active pharmaceutical ingredient	USP, Rockville, MD, USA
Citric acid, anhydrous, granular, USP	209.0	Solubilizer, stabilizer, pH reducer	Spectrum Chemical Manufacturing Corporation, New Brunswick, NJ, USA
Microcrystalline cellulose, NF	40.0	Disintegrant	Spectrum Chemical Manufacturing Corporation, New Brunswick, NJ, USA
Silicon dioxide	11.0	Disintegrant, anti-caking agent	Honeywell International Inc, Muskegon, MI, USA
Polyvinyl pyrrolidone	4.0	Binding agent, disintegrant	Sigma-Aldrich, St. Louis, MO, USA
Magnesium stearate, NF	1.5	Anti-caking agent	Spectrum Chemical Manufacturing Corporation, New Brunswick, NJ, USA
Total	**268.0**		

Table 2. Summary of plasma pharmacokinetic parameters for parent drug (pimobendan) and its active metabolite (o-desmethyl-pimobendan [ODMP]), generated using compartmental analysis, in seven healthy dogs administered 2.5 mg of pimobendan as a single oral dose of Vetmedin®, a citrate-containing experimental pimobendan capsule, or a citrate-free experimental pimobendan capsule. Geo Mean, geometric mean; Geo CV%, geometric coefficient of variation; K01, absorption rate, with associated half-life (T½); K10, terminal rate, with associated half-life (T½); AUC, area under the plasma drug concentration-time curve; T_{MAX}, time to peak plasma concentration; C_{MAX}, peak plasma concentration; Relative Abs, relative absorption compared to the Vetmedin® formulation determined by AUC ratios; Relative C_{MAX}, relative peak concentration compared to the Vetmedin® formulation determined by C_{MAX} ratios; ODMP (%) of pimobendan, metabolite-to-parent AUC ratio

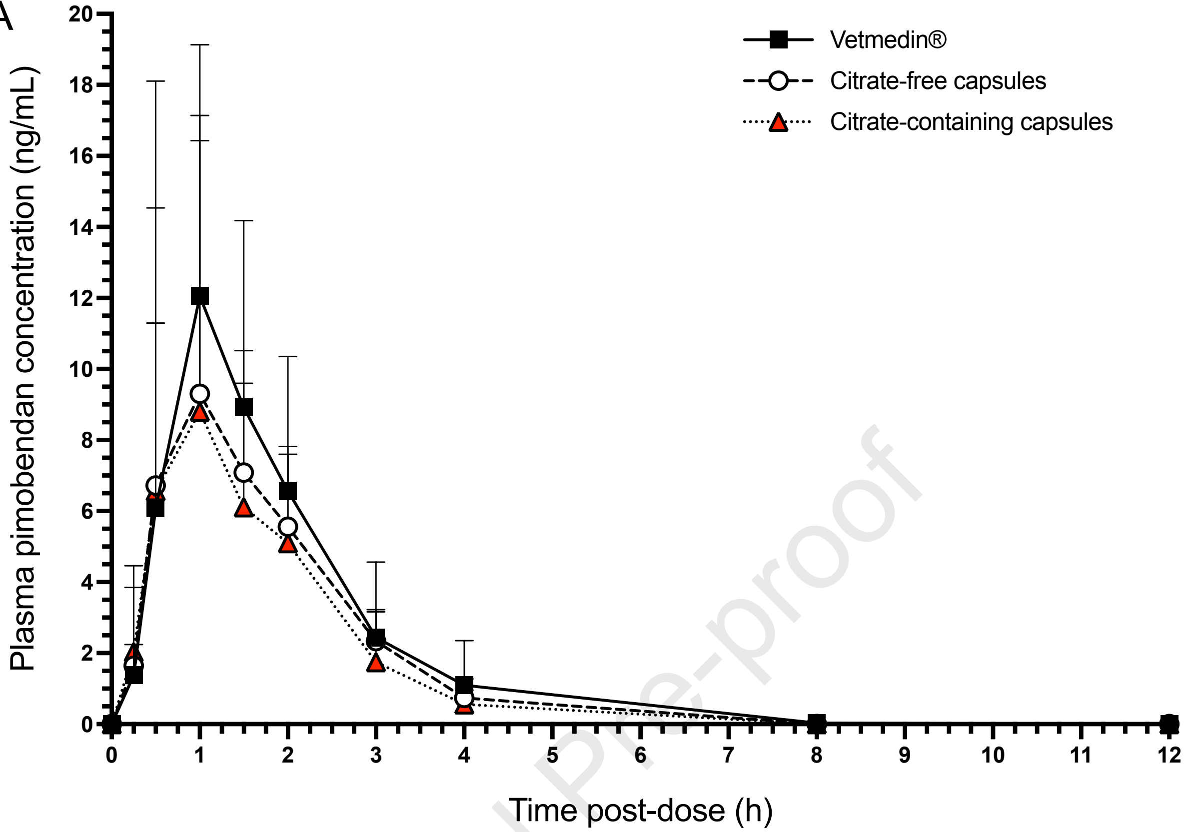
Parameter	**Vetmedin®**		**Citrate-containing capsule**		**Citrate-free capsule**	
	Geo Mean	Geo CV%	Geo Mean	Geo CV%	Geo Mean	Geo CV%
Parent compound (pimobendan)						
C_{MAX} (ng/mL)	8.82	52.6	5.85	76.0	8.11	70.2
T_{MAX} (hr)	0.92	20.8	0.92	51.7	0.95	55.1
AUC (hr*ng/mL)	19.0	54.9	13.6	68.0	19.7	40.4
K01 (1/hr)	2.23	69.7	1.31	66.4	1.16	29.3
K10 (1/hr)	0.93	45.6	1.07	36.2	1.09	38.5
K01 T½ (hr)	0.31	46.5	0.53	57.1	0.60	38.2
K10 T½ (hr)	0.74	82.8	0.65	51.5	0.64	77.7

Relative Abs (%)	-	-	71.5	61.4	104	71.1
Relative C_{MAX} (%)	-	-	66.3	58.5	91.9	95.3
Active metabolite (ODMP)						
C_{MAX} (ng/mL)	8.74	45.65	6.39	61.41	7.07	63.0
T_{MAX} (hr)	1.29	19.72	1.58	66.75	1.59	28.4
AUC (hr*ng/mL)	30.3	34.98	24.61	46.80	27.25	34.0
K01 (1/hr)	1.71	82.89	0.94	57.65	1.08	50.5
K10 (1/hr)	0.54	41.2	0.57	38.01	0.52	32.4
K01 T½ (hr)	0.41	59.29	0.74	82.12	0.64	52.2
K10 T½ (hr)	1.28	74.7	1.21	75.91	1.32	46.7
ODMP (%) of pimobendan	159	30.0	181	31.1	139	24.1

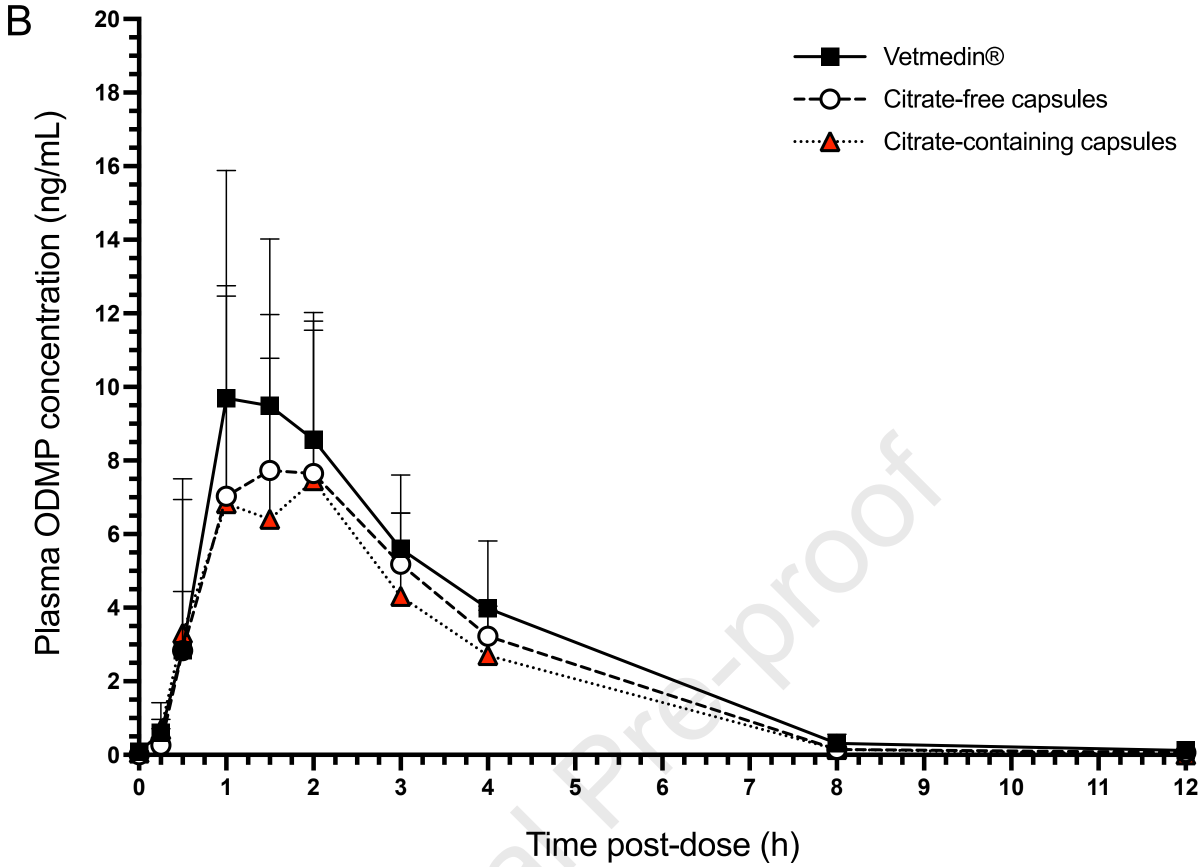
Table 3. Summary of published veterinary studies reporting information related to pharmacokinetic variability in healthy dogs administered pimobendan by mouth. -, information not listed/available

Formulation	Oral dose (mg/kg)	Number of dogs	Coefficient of variation (%)		Reference
			C_{MAX}	AUC	
Vetmedin® tablets (US)	0.25	-	25%	-	US product label
Vetmedin® tablets (US)	0.5	8	58.4%	48.2%	Her et al. 2020
Vetmedin® oral solution (US)	0.29 – 0.41	24	76.3%	67.9%	US product label
Vetmedin® capsules	0.25	10	49.9%	54.3%	Bell 2015
pimobendan liquid formulation 1			23%	48.2%	
Vetmedin® 3.5 mg/mL liquid solution	0.3	4	6%	23.6%	Saengklub 2022
pimobendan liquid formulation 2			18.7%	12.5%	
Pimobendan in PCCA fixed oil suspension™	0.1	8	31.5%	36.4%	Guth et al. 2015
	0.3		36.9%	46.2%	
	1.0		59.0%	19.8%	

A



Plasma pimobendan concentration (ng/mL)
Time post-dose (h)
Vetmedin®
Citrate-free capsules
Citrate-containing capsules
0
2
4
6
8
10
12
14
16
18
20
0
1
2
3
4
5
6
7
8
9
10
11
12



B
Plasma ODMP concentration (ng/mL)
Time post-dose (h)
Vetmedin®
Citrate-free capsules
Citrate-containing capsules
0
2
4
6
8
10
12
14
16
18
20
0
1
2
3
4
5
6
7
8
9
10
11
12