Oral pharmacokinetics of fenbendazole in llamas, 
South American Camelids

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Abstract

Llamas, South American Camelids are increasingly popular in the United States, as a source of fiber, livestock guard, and pack animals. Gastrointestinal parasites have been identified as a major health problem in all classes of livestock including llamas. Currently, there are no approved anthelmintics available for use in llamas. In this study, the pharmacokinetics of a single, oral administration of fenbendazole paste at a minimum target dose of 5 mg/kg, with an upper limit of <10 mg/kg, was evaluated in llamas. Plasma fenbendazole concentration time profiles were best described by a single compartment model. After oral administration of fenbendazole, $T_{\text{max}}$ and $C_{\text{pmax}}$ were 28.39$\pm$12.80 h, and 0.28$\pm$0.17 $\mu$g/ml, respectively. The $T_{1/2a}$ and $T_{1/2b}$ were 16.25$\pm$11.67 and 36.00$\pm$25.00 h, respectively. The apparent volume of distribution ($V_d$) and the area under the curve (AUC) were 11.28$\pm$4.66 l/kg, and 22.52$\pm$8.67 $\mu$g h/ml, respectively. The results of this preliminary study indicate that when the paste formulation of fenbendazole is administered orally to llamas, its rate of absorption appears to be very similar to that of other ruminants including sheep, goats, and cattle as indicated by the time required to reach peak plasma concentrations. It was also found that the rate of elimination of fenbendazole was prolonged in llamas as compared to sheep, goats, and cattle. © 2000 Elsevier Science B.V. All rights reserved.

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1. Introduction

Gastrointestinal parasites have been identified as a major health problem in livestock including llamas. Although there are significant numbers of ‘minor species’ animals including sheep, goats, llamas, and alpacas raised in the United States for food, fiber and companion purposes, there is a lack of drugs that are officially approved by the Food and Drug Administration for use in these animals. There are over 100,000 llamas, and 10,000 alpacas with smaller numbers of guanacos and vicunas in the United States by current estimates of the Llama Breeders Association (Oklahoma Llamas Breeders Association, 1998, personal communication). In Oklahoma alone, there are 45 breeding farms that are members of the
Oklahoma Llamas Breeders Association with numerous others that are not registered with the association (Breeder and Service Directory, 1998). Although little is known concerning the incidence of gastrointestinal parasitic diseases and their impact on the health of South American Camelfids (SACs), there is a significant need for identifying anthelmintic agents that are clinically effective and safe for use in these animals (Fowler, 1998). Fenbendazole is a member of the benzimidazole group of anthelmintics and has a broad spectrum of activity against gastrointestinal parasites, lungworms, and some tapeworms. This drug is currently approved and widely used in horses, dogs, pigs and cattle (Roberson, 1988). Fenbendazole has been studied extensively in sheep and goats, and is currently being recommended and used as an extra-label anthelmintic in llamas, alpacas and other SACs (Cheney and Allen, 1989; Fowler, 1998). The results of a recent study have shown that oral administration of a single dose of fenbendazole paste at 5 mg/kg was effective against naturally occurring gastrointestinal parasite infestations of Nematodirus, Strongyloides and Trichuris in llamas (Beier et al., 2000). It has been previously reported that the efficacy of benzimidazole anthelmintics is dependent upon their optimal plasma and tissue concentrations and the time during which the concentration is maintained (Sanyal, 1998). Therefore, the determination of pharmacokinetic parameters in any given species is a critical matter. To our knowledge, there are no studies available concerning the pharmacokinetic profiles of this drug in llamas. Therefore, the major objective of this study was to characterize oral pharmacokinetic profiles of fenbendazole in llamas.

2. Materials and methods

2.1. Animals

Five, healthy, young adult llamas of both sexes (three females and two males) weighing from 55 to 170 kg and purchased from local farms were used in this study. Each animal was assessed and found to be healthy and normal by physical examination, CBC, blood chemistry profiles, and urinalysis. No parasite eggs were observed based upon routine fecal examination. The llamas were individually housed at the University Lab Animal Resources barn in accordance with the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, 1996). Drinking water and a balanced ration of grain and prairie hay were provided ad libitum during this study.

2.2. Oral administration of fenbendazole and collection of blood samples

A group of five llamas was used in this experiment. Using chute restraint, aseptic surgical technique, and a local anesthetic, indwelling catheters were placed in the right jugular veins of individual llamas. At time 0, 24 h later, fenbendazole (Safe-Guard® paste, Hoechst–Roussel Agri-Vet, Somerville, NJ) at a minimum target dose of 5 mg/kg was orally administered to each animal using a dispensing gun. This instrument (Hoechst–Roussel Agri-Vet, Somerville, NJ) has been designed so that each full depression of the dispensing gun trigger delivers approximately 500 mg of fenbendazole. This 500 mg dose is sufficient to treat animals weighing up to 220 lb (100 kg) body weight, based upon label information for approved species (cattle). In accordance with manufacturer’s directions for treating approved species and instructions to not underdose the product, llamas weighing from 55 to 100 kg received 500 mg of fenbendazole and those weighing more than 100 kg and up to 170 kg received 1 g of fenbendazole, with calculated doses ranging from 5 mg to <10 mg/kg. The drug was administered at approximately, 14 h after feeding the animal with a balanced grain ration. There was no regurgitation or spitting observed in each of the five animals after the administration of the drug. Blood samples (5 ml) were collected in a heparinized syringe via the indwelling intravenous catheter at 0, 15, 30, and 45 min and 1, 2, 3, 4, 6, 8, 10, 12, 18, 24, 36, 48, 120 and 144 h periods. Following sample collection, 5 ml of normal saline was injected to replace the volume of the blood sample collected. Blood samples were centrifuged at 2000 × g for 10 min; plasma samples were collected and stored at −20°C until analyzed.

2.3. Chemicals and standard solutions

All reagents used in this study were HPLC grade. Deionized distilled water (Milli-Q water system,
Millipore Corp., Bradford, MA, USA) was used throughout the study. Analytical (HPLC) grade methanol, acetonitrile, chloroform, and ammonium phosphate were obtained from Fisher Chemicals (Fair Lawn, NJ, USA). Stock solutions of fenbendazole and its internal standard, oxibendazole, (Sigma Chemical Co., St. Louis, MO, USA) were prepared in methanol at concentrations of 0.25 and 0.1 mg/ml and then stored at −20°C. The stock solutions were diluted further to yield working solutions for the preparation of HPLC standards as needed.

2.4. Extraction procedure

For determination of fenbendazole, 10 μl of oxybendazole (10 μg/ml) was added to 500 μl of plasma as the internal standard. Chloroform (7 ml) was then added, and the mixture was vortexed thoroughly for 3 min. Following centrifugation (2000×g, 5 min), the organic phase was transferred into a clean test tube after the aqueous layer was frozen under acetone/dry ice conditions. The chloroform extracts were evaporated to dryness under a slow steady stream of nitrogen. The resulting residue was reconstituted in 100 μl of methanol, and an aliquot of 50 μl of this solution was injected into the HPLC for analysis. The extraction recovery of fenbendazole and oxybendazole from llama plasma in this study was 68.4±3.3% and 68.4±1.67%, respectively.

2.5. HPLC determination of plasma fenbendazole

The HPLC analysis of plasma fenbendazole was carried out using the Waters HPLC system (Waters, Millipore Co., Milford, MA, USA). This unit was comprised of a pump (Model 501), a tunable absorption detector (Waters 484), and chromatographic software (Maxima 820). The analytical Lichrospher 100 (Hewlett Packard, Inc., Wilmington, DE, USA) RP-18 column (5 μm, 250×4 mm) and a guard column, Lichrospher 100, RP-18 (5 μm, 4×4 mm), were used to resolve fenbendazole and oxibendazole (internal standard), respectively.

The mobile phase consisting of acetonitrile:10 mM ammonium phosphate buffer (pH 3.7, 48:52, v/v) degassed, using a vacuum bottle and pump at −15 psi (−103 kPa) was used. The column was equilibrated and eluted under isocratic conditions utilizing a flow rate of 1.0 ml/min at ambient temperature. The detection wavelength was set at 292 nm. The peak width, response time, and slit were set at >0.03 min, 0.5 s, and 8 nm, respectively. Standard curves were prepared daily by comparing peak area ratios of fenbendazole concentrations in plasma to peak area ratios of the internal standard.

Method validation was performed by comparing peak area ratios of fenbendazole:oxybendazole (internal standard) from a range of standard solutions in drug-free llama plasma. Additionally, precision was determined by comparing the coefficients of variation of inter-day variations in the peak area ratios of the standard curves. The results of the variations indicated that the HPLC method was suitable for pharmacokinetic studies of fenbendazole in llamas with an accuracy of 94±8%, coefficient of variation of 10%, and detection limit of quantitation of 0.01 μg/ml.

2.6. Determination of the pharmacokinetic parameters of orally administered fenbendazole

Curve fitting and pharmacokinetic parameter calculations were carried out by estimation using the Boomer program (Bourne, 1989), and by analysis using the PharmK program (Lu and Mao, 1993). An appropriate pharmacokinetic model was chosen on the basis of lowest weighted squared residuals, lowest Akaike’s information criterion (AIC) value, R-squared, and correlation coefficient (Akaike, 1974). The area under the curve (AUC) was calculated by the trapezoidal rule between 0 h and the last sampling time plus Cp/β, where Cp is the plasma concentration of the last sampling and β is the elimination rate constant (Gibaldi and Perrier, 1975). The time (Tmax) taken to achieve peak concentration (Cpmax) was calculated using differential calculus (Gibaldi and Perrier, 1975). The results are presented as means and standard errors of the mean (SEM).

3. Results and discussion

3.1. Analysis of fenbendazole

Several combinations of acetonitrile:10 mM ammonium phosphate buffer (pH 3.7) were evaluated as possible mobile phases. It was determined that a ratio
of acetonitrile:10 mM ammonium phosphate buffer (pH 3.7, 48:52, v/v) was most suitable for separation of fenbendazole. Changing the ratios of acetonitrile and ammonium phosphate buffer alters the retention time of fenbendazole. Larger areas yielded shorter retention times, which caused interference by other peaks produced by the methanol solvent.

3.2. Oral pharmacokinetic parameters

The mean plasma fenbendazole concentration–time curve over 120 h for oral route of administration is presented in the main portion of Fig. 1. The inset at the top of Fig. 1 represents the mean plasma fenbendazole concentrations more clearly for the first 24 h following oral administration compared to the main figure. The concentration range of plasma was from 0.025 to 0.283 μg/ml after a targeted minimum oral dose of 5 mg/kg of fenbendazole. The plasma concentration of fenbendazole concentration data was best fitted to the following exponential equation:

\[ C_p = A e^{-\alpha t} + B e^{-\beta t} \]

In the preceding equation, \( C_p \) is plasma fenbendazole concentration; \( A \) and \( B \) represent mathematical coefficients; \( \alpha \) and \( \beta \) represent the hybrid rate constants for the distribution and terminal elimination phases, respectively. Various pharmacokinetic parameters including absorption (\( T_{1/2a} \)) and elimination (\( T_{1/2b} \)) half-lives, volume of distribution (\( V_d \)), maximal plasma concentration (\( C_{pmax} \)) and the time to

Fig. 1. (bottom): Mean plasma concentration time curve (over 120 h) of fenbendazole paste in llamas (\( n=5 \)) following oral administration of a single dose at 5 mg/kg. The inset (top) more clearly illustrates changes in the plasma concentration during the first 24 h following fenbendazole administration.
Table 1
Pharmacokinetic parameters following oral administration of fenbendazole paste (5 mg/kg) to llamas (n=5)\(^a\)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value (mean±SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\alpha) (h(^{-1}))</td>
<td>0.07±0.06</td>
</tr>
<tr>
<td>(\beta) (h(^{-1}))</td>
<td>0.025±0.01</td>
</tr>
<tr>
<td>(T_{1/2a}) (h)</td>
<td>16.25±11.67</td>
</tr>
<tr>
<td>(T_{1/2b}) (h)</td>
<td>36.00±25.00</td>
</tr>
<tr>
<td>(V_d) (l/kg)</td>
<td>11.28±4.66</td>
</tr>
<tr>
<td>(C_{p_{max}}) (µg/ml)</td>
<td>0.28±0.17</td>
</tr>
<tr>
<td>(T_{max}) (h)</td>
<td>28.39±12.80</td>
</tr>
<tr>
<td>AUC (µg h/ml)</td>
<td>22.52±8.67</td>
</tr>
</tbody>
</table>

\(a\) Abbreviations: \(\alpha\)=absorption rate constant; \(\beta\)=elimination rate constant; \(T_{1/2a}\)=absorption half-life; \(T_{1/2b}\)=elimination half-life; \(V_d\)=apparent volume of distribution calculated using AUC; \(C_{p_{max}}\)=maximal plasma concentration; \(T_{max}\)=time to achieve \(C_{p_{max}}\); AUC=area under the curve.

achieve maximal plasma concentration (\(T_{max}\)) were calculated for individual llamas and the mean of above parameters for a group of five animals are presented in Table 1.

Following oral administration of fenbendazole in llamas, the drug reached peak concentration of 0.28±0.17 µg/ml in the blood at approximately 28 h and dropped to a concentration below the detectable level at 140 h. The peak plasma concentration (0.28 µg/ml) of fenbendazole in llamas is approximately two-thirds of the reported value (0.40 µg/ml) for sheep (Lanusse et al., 1995). However, it is about twice the value of goats (0.13 µg/ml) and cattle (0.15 µg/ml) (Short et al., 1987a, b; Lanusse et al., 1995). The time of 28 h necessary to achieve peak plasma concentration in llamas is comparable that of sheep (20 h), goats (24 h) and approximately twice that of cattle (12 h) (Short et al., 1987a, b; Lanusse et al., 1995). However, the elimination time (120 h±0.25) is significantly longer in llamas compared to those of sheep (70 h), goats (73 h) and cattle (96 h) (Short et al., 1987a, b; Sanyal, 1993; Lanusse et al., 1995).

4. Conclusions

The results of this preliminary pharmacokinetic study indicate that when fenbendazole is administered orally in the paste formulation, its rate of absorption is similar that of other ruminants, including sheep, goats, and cattle, as indicated by the time required to reach peak plasma concentrations. In the absence of pharmacokinetic parameters including the area under the curve following an intravenous dose of fenbendazole under identical experimental conditions, the rate and extent of fenbendazole absorption (bioavailability) was not determined and is unknown in llamas at this time. It was also found that the rate of elimination of fenbendazole was prolonged in llamas compared to sheep, goats, and cattle. This may be partly due to slower hepatic metabolism of the drug or its large volume of distribution or both. These pharmacokinetic properties were consistent with the observation that fenbendazole, when administered orally at a single minimum dose of 5 mg/kg, was very effective clinically for a period of 4 weeks in llamas with naturally occurring gastrointestinal parasitism (Beier et al., 2000).

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