FENBENDAZOLE PHARMACOKINETICS, METABOLISM, AND POTENTIATION IN HORSES

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(Received February 5, 2002; accepted July 22, 2002)

ABSTRACT:

The present study was designed to describe the pharmacokinetics and fecal excretion of fenbendazole (FBZ) and fenbendazole sulfoxide (FBZSO) and their metabolites in horses, to investigate the effects which concurrent feeding has on the absorption and pharmacokinetics of FBZ, and to determine the effect of coadministration of the metabolic inhibitor piperonyl-butoxide on the in vivo pharmacokinetics and in vitro liver microsomal metabolism of sulfide and sulfoxide benzimidazoles. The effect of piperonyl-butoxide on the enantiomeric genesis of the sulfoxide moiety was also investigated. Following administration of FBZSO and FBZ, the fenbendazole sulphone metabolite predominated in plasma, and the Cmax and area under the plasma curve (AUC) values for each moiety were larger (P < 0.001) following FBZSO than FBZ. In feces the administered parent molecule predominated. The combined AUC for active benzimidazoles moieties following oral administration of FBZ (10 mg/kg) in horses was almost 4 times as high in unfed horses (2.19 μg·h/ml) than in fed horses (0.59 μg·h/ml), and coadministration of piperonyl-butoxide significantly increased the AUC and Cmax of active moieties following intravenous administration of FBZSO and oral administration of FBZ. When FBZSO was administered i.v. as a racemate, the first enantiomer of oxfendazole (FBZSO-1) predominated in plasma, however, following coadministration with piperonyl-butoxide, the second enantiomer of oxfendazole (FBZSO-2) predominated for 10 h. Piperonyl-butoxide significantly reduced the oxidative metabolism of FBZSO and FBZ in equine liver microsomes and altered the ratio of enantiomers FBZSO-1/FBZSO-2 from >4:1 to 1:1. It is concluded that in horses efficacy of FBZSO and FBZ could be improved by administration to unfed animals and coadministration with piperonyl-butoxide.

Helminth parasites produce pathological changes in the horse including diarrhea (Mair et al., 1990), rapid progressive weight loss (Love, 1992), functional disorders of the intestine (Ogbourne and Duncan, 1977), and colic and pathological changes in the mesenteric arteries (Duncan and Dargie, 1975).

Large strongyles (Strongylus vulgaris, Strongylus edentatus, Strongylus equinus) migrate from the gastrointestinal tract through viscera and blood vessels where orally administered anthelmintics with low bioavailability are ineffective. The small strongyles (Cystothostominae) may become inhibited within the large intestinal mucosa at the early third and fourth larval stages, which are largely recyclant to available anthelmintics. The selection of anthelmintic resistant populations has increased since phenothiazine resistance was first recognized in horses in 1961 (Drudge and Elam, 1961). Selection of resistance to anthelmintics has developed rapidly in the horse (Love et al., 1989) probably because the epidemiology of equine parasites is less seasonal than that of ruminant parasites. Horses are consequently treated frequently throughout the year even in temperate climates thus exerting great selection pressure.

The pharmacokinetics of benzimidazole anthelmintics has been studied in horses in which the metabolic interconversion of the sulfide and sulfoxide benzimidazoles [fenbendazole (FBZ1)] and fenbendazole sulfoxide (FBZSO), respectively] seems to differ substantially from that in ruminants (Marriner and Bogan, 1985). Sulfide and sulfoxide benzimidazoles are known to bind nematode tubulin (Lacey et al., 1987) and therefore have activity against nematodes, although sulfides generally exert inhibitory activity on tubulin at lower concentrations than sulfoxides. In most species examined, the sulfoxide moiety predominates in plasma and is thought to confer activity against gut-dwelling nematodes following secretion across the gastrointestinal wall into the gut lumen where it may undergo sulfreduction.

The absorption and consequent efficacy of benzimidazoles is known to be influenced by their administration with food. The bioavailability of sulfide benzimidazoles is markedly reduced in ruminants which have had unrestricted access to food compared with those given restricted access prior to treatment. This is thought to be associated with adsorbence on to, and passage of, particulate associated drug in the digestive tract (Hennessy et al., 1994). The opposite is true in

1 Abbreviations used are: FBZ, fenbendazole; FBZSO, fenbendazole sulfoxide (oxfendazole); DMSO, dimethyl sulfoxide; FBZSOen, fenbendazole sulphone; OH-FBZ, hydroxy-fenbendazole; HPLC, high performance liquid chromatography; FBZSO-1, the first enantiomer of oxfendazole; FBZSO-2, the second enantiomer of oxfendazole; AUC, area under the plasma concentration time curve; AUMC, area under the moment curve; MRT, mean residence time; r, recovery; FMO, flavin-containing monoxygenase.
the dog (McKellar et al., 1993) in which coadministration with food increases the bioavailability of the benzimidazole by up to three times.

The coadministration of metabolic inhibitors has been used to reduce the rate of metabolic oxidation of benzimidazole sulfides and sulfoxides to sulfones, which are inactive and thus improve their efficacy (Hennessy et al., 1985, Lanusse and Prichard, 1991). Piperonyl-butoxide is a potent inhibitor of cytochrome P450 oxidation, which has been shown to alter the pharmacokinetic profile of FBZ and to potentiate its nematocidal activity in sheep (Benchouai and McKellar, 1996). The potential for improved efficacy of benzimidazoles coadministered with inhibitors of oxidative metabolism is greater in the horse than in ruminants since the rate of oxidative metabolism and thus inactivation of sulfoxide moieties in the horse seems to be extremely rapid (Marriner and Bogan, 1985).

The excretion of anthelmintics in the feces of livestock has given rise to concern since it was observed that the avermectins have adverse effects in dipteran flies and coleopteran beetles, which inhabit and feed in dung (Wall and Strong, 1987). Whereas it is recognized that benzimidazoles are unlikely to affect dung-dwelling arthropods (McKellar, 1997), environmental impact is not limited to specific effect on scavenger insects, and their excretion in the faeces of horses has not been characterized, and consequently the associated environmental impact is not known.

The present studies were designed to describe the pharmacokinetics and fecal excretion of FBZ and FBZSO and their metabolites in horses, to investigate the effects which concurrent feeding has on the absorption and pharmacokinetics of FBZ, and to determine the effect of coadministration of the metabolic inhibitor piperonyl-butoxide on the in vivo pharmacokinetics and in vitro equine liver microsomal metabolism of sulfide and sulfoxide benzimidazoles. The effect of piperonyl-butoxide on the enantiomeric genesis of the sulfoxide moiety was also investigated.

Materials and Methods

Animals and Experimental Design. Oral pharmacokinetic and fecal excretion study. Sixteen horses of mixed breed weighing 390 to 720 kg were allocated in a restricted random fashion to two groups of eight, which were balanced for weight. Animals were kept under field conditions with unrestricted access to herbage and water. They were only corralled for the period of drug administration, and all other procedures were carried out with minimal restraint in the field. Commercially available formulations of FBZ (Panacur, 18.75% w/w; Hoechst Pharmaceutical Research Labs, Milton Keynes, Bedfordshire, UK) and oxendazole [FBZSO] (Systemax 906, 9.06%/w/v; Mallinckrodt Veterinary Ltd., Uxbridge, UK) were administered orally as a single bolus dose on the back of the tongue at 10 mg/kg bodyweight. Heparinized blood samples (approximately 10 ml) were collected by jugular venepuncture and fecal samples (>10g) were collected per rectum at predetermined times until 120 h after drug administration.

Intravenous enantioselective pharmacokinetics of oxendazole alone and following coadministration with piperonyl-butoxide. Seven ponies weighing 164 to 250 kg were randomly allocated to two groups comprising four and three animals. Ponies were kept indoors and had hay and water available ad libitum throughout the course of the study. Oxendazole [FBZSO] 99.9%; Schering-Plough, Uxbridge, UK] was prepared in dimethyl sulfoxide (DMSO, 500 mg/ml; Sigma-Aldrich, Gillingham, UK) and administered at a dose rate of 10 mg/kg bodyweight by right jugular venepuncture to one group of ponies. The other group was administered piperonyl-butoxide [90%; Sigma-Aldrich] by nasogastric intubation at a dose rate of 31 mg/kg bodyweight and 30 min later was administered FBZSO as described above. Heparinized blood samples were collected by jugular venepuncture from the left jugular vein at predetermined times until 96 h after drug administration. After a 4 week washout period, the experiment was repeated with the groups reversed according to a two-phase crossover design.

Oral pharmacokinetics of fenbendazole administered with and without food and following coadministration with piperonyl-butoxide. Six ponies weighing 94 to 216 kg were randomly allocated to two groups of three. They were kept indoors and had hay and water available ad libitum except for the period immediately (for 12 h) prior to each drug administration when the hay and any straw bedding were removed. Three animals were administered FBZ (Panacur 2.5% w/w; Hoechst Pharmaceutical Research Labs) orally as a single dose by nasogastric intubation at 10 mg/kg bodyweight. Three animals received FBZ as above 30 min after receiving a 63 mg/kg dose of piperonyl-butoxide also by nasogastric intubation. This study was repeated after a 4-week washout period with the groups reversed. Animals were fed four h after drug administration. Three months after the above study was completed, it was repeated identically except that the horses were given approximately 0.5 kg of cereal concentrates 1 h before drug administration and a further 0.5 kg just before drug administration. Heparinized blood samples were collected by jugular venepuncture at predetermined times until 96 h after drug administration. All animals were given access to hay ad libitum 4 h after FBZ administration.

Oral administration of fenbendazole and oxendazole to a horse with a chronic cecal fistula. A pony weighing 260 kg with a chronic cecal fistula, which had been inserted by the method of Boyd (1985) was used. Fenbendazole and FBZSO were administered orally and intra cecally at a dose of 10 mg/kg with a week washout period between drug administrations. Blood was collected by jugular venepuncture at predetermined times (identical to those used for the oral administration studies). All analytical methods were the same as those used in the above oral studies.

The effect of piperonyl-butoxide on the metabolism and chirality of benzimidazoles in micromoses from equine liver. The livers were collected from seven horses being euthanized for clinical reasons unrelated to hepatic disease. After the horse was killed, its liver was removed and perfused with ice-cold saline (0.9% NaCl solution) through the hepatic veins. A portion of liver was dissected free, drained of excess moisture, and weighed to make 100 g for each animal.

Drug Analysis. Blood samples were centrifuged at 1825g for 30 min and plasma was transferred to plastic tubes. All the plasma and fecal samples were stored at −20°C until estimation of drug concentration. Plasma and wet-fecal concentration of FBZ, FBZSO, FBZSO, and hydroxy-fenbendazole (OH-FBZ) were estimated by high performance liquid chromatography (HPLC) with a liquid phase extraction procedure adapted from that of Marriner and Bogan (1980). Standard compounds of FBZ, FBZSO, FBZSO, and OH-FBZ were obtained from Hoechst (Frankfurt, Germany).

Drug-free plasma samples (1 ml) were spiked with standards of FBZ, and its metabolites (FBZSO, FBZSO, and OH-FBZ) for the FBZ study, FBZSO and its metabolites (FBZ, FBZSO, and OH-FBZ) for the FBZSO study, and oxibendazole as an internal standard to reach the following final concentrations: 0.01, 0.05, 0.1, 0.5, and 1 µg/ml. Ammonium hydroxide (200 µg/ml, 0.1 N, pH 10) was added to 10 ml-ground glass tubes containing 1 ml of spiked or experimental plasma samples. After vortexing for 15 s, 6 ml of chloroform (Rathburn Chemical Ltd., Walk barner, Scotland, UK) was added. The tubes were shaken on a slow rotary mixer for 10 min. After centrifugation at 1825g for 15 min, the supernatant was removed with a Pasteur pipette. The organic phase (4 ml) was transferred to a thin-walled 10 ml-conical glass tube and evaporated to dryness at 43°C in a sample concentrator (model SC10A; Savant Instrument Inc., Holbrook, NY). The dry residue was resuspended with 50 µl of DMSO and 200 µl of 25% acetonitrile. The tubes were placed in an ultrasonic bath and 50 µl of this solution was injected into the chromatographic system. Wet fecal samples were mixed finely with a spatula to obtain homogeneous concentrations. Drug-free wet feces samples (0.5 g) were spiked with benzimidazole standards, described above, to reach the following final concentrations: 1, 5, 50, 100, 200 µg/g. Sodium hydroxide buffer (200 µl, 0.4M, pH 10) and 2 ml of acetonitrile were added to 10 ml-ground glass tubes containing 0.5 g of spiked or experimental wet-fecal samples. After vortexing for 15 s, 8 ml of chloroform was added. The tubes were shaken on a slow rotary mixer for 15 min. After centrifugation at 1825g for 15 min, the supernatant was removed with a Pasteur pipette. The organic phase (5 ml) was transferred to a thin-walled 10 ml-conical glass tube and evaporated to dryness at 43°C in the sample concentrator. The dry residue was resuspended with 50 µl of DMSO and diluted appropriately with 35% acetonitrile. After ultrasonication, the samples were filtered with glass microfibre filter (Whatman International Ltd, Maidstone, Kent, UK) Finally, 50 µl of this solution was injected into the chromatographic system.
The HPLC system (PC1000; Spectra Physics Analytical Inc., Manchester, UK) comprised a gradient pump (model SP4000), a UV-detector (SP Focus) set at 292 nm, an autosampler (model AS 3000), and a controller (model SN 4000). The mobile phase was a mixture of acetonitrile-water to which glacial acetic acid was added (0.5% v/v). It was pumped through the column (Genesis nucleosil C<sub>18</sub> 4 μm, 150 mm × 4.6 mm; Crawford Scientific, Strathaven, Lanarkshire, Scotland, UK) at a flow rate of 1 ml/min in a linear gradient from 35:65 (acetonitrile-water) to 40:60 for 9 min, 60:40 to 35:65 for 1 min and 35:65 for 2 min. The retention times were 5.5 (FBZSO), 6.8 (OH-FBZ), 7.4 (FBZSO<sub>2</sub>), and 10.2 (FBZ) min (Fig. 1). Recovery of parent molecules was determined by comparison of peak areas on chromatograms from spiked plasma and fecal samples with areas from direct injection of standards. The interassay precision was evaluated by processing replicate aliquots of spiked horse plasma or feces on different days.

For chiral analysis of FBZSO, plasma samples were extracted as described above and the residue resuspended with 50 μl of DMSO and 150 μl of H<sub>2</sub>O, 50 μl of which was injected into the chromatographic system. A mobile phase of acetonitrile/water (7:93) was pumped at a flow rate of 0.9 ml/min through a chiral-AGP column (5 μm, 100 × 40 mm; BAS Instruments Ltd., Congleton, Cheshire, UK) with ultraviolet detection at 296 nm. Retention times of the enantiomers were 7.87 min for FBZSO-1 and 10.43 min for FBZSO-2.

For analysis of microsome incubation mixture, the whole sample was recovered and the tube washed with 1 ml of acetonitrile. Chloroform (6 ml) was added and the tube shaken on a slow rotary mixer for 10 min. The tube was then centrifuged at 182g for 15 min and two 3 ml of aliquots collected for achiral and chiral analysis. The HPLC systems were as described above, but for achiral analysis the gradient profile of the mobile phase changed from 25:75 (acetonitrile-water) to 45:55 in 6 min, to 75:25 in 11 min and then to 25:75 for 13 min. The flow rate was 1.5 ml/min, and the retention times were 3.48 min (FBZSO), 4.68 min (OH-FBZ), 5.58 min (FBZSO<sub>2</sub>), and 8.41 (FBZ). Analysis of fecal samples was carried out on a wet weight basis, and a 10 g sample of feces taken and dried in an oven at 70°C for 2 min to terminate the reaction then immediately stored at −20°C until analysis.

Liver Microsome Preparations. One hundred grams of finely chopped liver tissue was placed in 300 ml of 1.15% KCl solution and homogenized using a Potter-Elvehjem homogenizer (Cambridge, UK). The homogenate was centrifuged for 20 min at 9000g to remove debris, nuclei, and mitochondria. The surface fat layer was removed and the supernatant decanted and further centrifuged at 105,000g for 75 min in a Beckman L8–70 refrigerated ultracentrifuge (Beckman Coulter Ltd., High Wycombe, Buckinghamshire, UK). The cytosolic supernatant was removed and the microsomal pellet resuspended in 60 ml of 0.1 M tris-phosphate buffer (pH 7.4) containing 20% (v/v) glycerol. The protein content of the microsomal suspensions were determined using the Coomassie blue protein assay reagent.

Drug incubations were carried out in triplicate in a shaking-water bath of 37°C for 1 h. Glass test tubes (10 ml) were used. One assay of an incubation mixture contained 4 mg of microsomal protein, 5 μl of test drugs (0.5, 1, and 2.5 μM of FBZSO, FBZ, and OBZ standards were each dissolved in DMSO) alone or with 5 μl of piperonyl-butoxide and 1 ml of cofactor solution. Piperonyl-butoxide was used at a constant concentration of 5 μM. Tubes without microsome were used as controls for possible nonenzymatic drug conversion. After incubation the tubes containing reaction mixture were placed in boiling water for 2 min to terminate the reaction then immediately stored at −20°C until analysis.

Pharmacokinetic and Statistical Analysis. The plasma concentration time curves obtained after each treatment in individual animals were fitted with the WinNonLin software program (Statistical Consulting Inc. Pharsight Corpora-
cion, Cary, NC). For all oral drug administration studies, noncompartmental model analysis with extravascular input was used. The <i>C</i><sub>max</sub> and time to reach <i>C</i><sub>max</sub> (T<sub>max</sub>) were obtained from the plotted concentration time curve for each moiety in each animal. The linear trapezoidal rule was used to calculate the area under the plasma concentration time curve (AUC) and area under the first moment curve (AUMC). The mean residence time (MRT) was determined as AUMC/AUC.

The pharmacokinetic data are reported as mean ± S.E.M., and pharmacokinetic parameters for each drug moiety obtained following intravenous and following oral administration were statistically compared using the Mann-Whitney U test. The Wilcoxon sign rank test was used to determine the statistical differences associated with feeding. In the microsomal incubation studies, the extent of conversion and amount of unchanged drug with and without metabolic inhibition were compared by one-way analysis of variance. Results were considered significant when P < 0.05.

Results

Analytical Methods. The percentage recoveries from plasma and interassay coefficients of variation were determined for FBZ, FBZSO,
and FBZSO₂ over the concentrations 0.05, 0.10, 0.25, 0.50, and 1.00 µg/ml. The mean (n = 40 for each) recovery (r) and CV were FBZ r = 84.16%, CV = 9.05; FBZSO r = 94.61%, CV = 9.44; and FBZSO₂ r = 93.51%, CV = 6.23. The limit of detection of the assays was 0.005 µg/ml for plasma and 0.2 mg/g for fecal samples. During chiral analysis the limit of detection for both FBZSO enantiomers was 0.02 µg/ml, and recoveries were 85.28% (CV = 7.8) and 86.69% (CV = 7.4%) for FBZSO-1 and FBZSO-2, respectively. OH-FBZ was not detected in plasma following either FBZ or FBZSO administration.

Oral Pharmacokinetic and Fecal Excretion Study. The plasma concentration time curves for FBZ, FBZSO, and FBZSO₂ following oral administration of FBZSO and FBZ are shown in Figs. 2 and 3, respectively. The pharmacokinetic data associated with each of these drug administrations are given in Table 1.

Following administration of FBZSO and FBZ as parent drugs, the FBZSO₂ metabolic moiety predominated in plasma. The AUC ratio of sulfoxide/sulfide/sulfone was approximately 3:1:9 following FBZSO administration and 1:4:7 following FBZ administration. The Cₘₐₓ and AUC values for each moiety were significantly (P < 0.001) larger following administration of FBZSO than FBZ, and the two known active moieties achieved approximately 25.6 times (FBZSO) and 2.3 times (FBZ) the AUC values following administration of FBZSO than FBZ as the parent compound. The residence times of the two active moieties were longer (but not significantly longer) following administration of FBZSO than FBZ but that of the sulfone metabolite was very similar following each administration (15.45 ± 2.10 h versus 16.50 ± 1.00 h, respectively).

The fecal concentration time curves for FBZ, FBZSO, and FBZSO₂ following oral administration of FBZSO and FBZ are shown in Figs. 4 and 5, respectively. Following each administration, no drug could be detected in feces for at least 12 h, and the maximal mean concentration occurred at 24h. Concentrations had declined to below the limit of analytical detection in most samples by 72 h after administration. In feces the parent molecule predominated and whereas the sulfide was present at a concentration of 0.21 mg/g following administration of the sulfoxide, the more oxidized metabolites never exceeded 0.017 mg/g in feces following FBZ administration.

Intravenous Enantioselective Pharmacokinetics of FBZSO Alone and following Coadministration with Piperonyl-butoxide. The plasma concentration time curves of FBZSO, FBZ, and FBZSO₂, respectively, following administration of FBZSO alone or in combination with piperonyl-butoxide are given in Figs. 6, 7, and 8, and pharmacokinetic data are presented in Table 2. When FBZSO was administered alone, the FBZSO moiety did not display a typical exponential decline over time. After an initial decline phase lasting approximately 45 min, the concentrations of FBZSO plateaued at between 0.94 and 1.5 µg/ml until approximately 12 h from which time concentrations declined to the limit of detection by 96 h. The concentrations of FBZSO following administration of FBZSO plus piperonyl-butoxide declined from administration for 30 min then increased until 7 h by which time the plasma concentration was 3.69 ± 0.50 µg/ml. Concentrations decreased from this peak until by 30 h, they were lower than concentrations following administration of FBZSO alone. The AUC and Cₘₐₓ of FBZSO were significantly larger (P < 0.01) when FBZSO was administered with piperonyl-butoxide than on its own.

The concentration of FBZ following administration of FBZSO alone or in combination with piperonyl-butoxide displayed a similar pattern to those of the parent molecule except that the maximal concentrations were achieved later (at 16 h) compared with those of FBZSO (7 or 8 h). Concentrations of FBZ were lower than those of FBZSO, and the AUC and Cₘₐₓ of FBZ were significantly (P < 0.01) lower following administration of FBZSO alone compared with administration of FBZSO with piperonyl-butoxide.

The concentration of FBZSO₂ increased to 1.49 ± 0.12 µg/ml at 10 h following administration of FBZSO alone. When FBZSO was given in combination with piperonyl-butoxide, a similar maximal mean concentration was achieved (1.46 ± 0.13 µg/ml), but it did not occur until 16 h after administration. The AUC ratios for sulfide/sulfoxide/sulfone were 33:1:46 following FBZSO alone and 21:1:19 following FBZSO in combination with piperonyl-butoxide.

The plasma concentration time curves of the enantiomers of FBZSO following administration of FBZSO (as a racemate) either alone or in combination with piperonyl-butoxide are shown in Fig. 9, and the ratios (as percentages) of each enantiomer are given in Fig. 10. When FBZSO (racemate) was administered alone, the FBZSO-1 enantiomer predominated in plasma from 45 min after drug administration. From approximately 5 h after administration until 24 h after administration, the ratio was approximately 60:40 in favor of FBZSO-1. Between 24 h and 48 h the ratio changed toward that of a racemate.

The coadministration of piperonyl-butoxide had a dramatic effect on the absolute and relative concentrations of each enantiomer. The FBZSO-2 enantiomer predominated from the time of administration...
until 10 h approaching a 60:40 ratio at 5 h after administration. From 10 h the FBZSO-1 enantiomer predominated, and the ratio FBZSO-1:FBZSO-2 increased until 30 h after administration and then decreased until 48 h at which time it approached a racemate.

**Oral Pharmacokinetics of Fenbendazole Administered with and without Food and following Coadministration with Piperonyl-butoxide.** There were no statistically significant differences in the pharmacokinetic parameters of the benzimidazole moieties when FBZ was administered with or without food. Nevertheless the total-combined AUC for the active benzimidazole moieties (FBZSO and FBZ) was almost 4 times as high in unfed horses (2.19 µg · h/ml) than in fed horses (0.59 µg · h/ml). The coadministration of piperonyl-butoxide significantly ($P < 0.05$) increased the AUC and $C_{\text{max}}$ of FBZ in fed and unfed horses. In fed horses piperonyl-butoxide coadministration increased the AUC of the active moieties FBZSO and FBZ by 13.9...
times and 13.2 times, respectively. In unfed horses the piperonyl-butoxide increased the AUC of the parent FBZ by 11 times but decreased the AUC of the FBZSO moiety by 2.3 times. The effect on combined active moieties was an increase in AUC of 2.0 times when FBZ was given with piperonyl-butoxide.

The Absorption of Fenbendazole and Oxfendazole following Intracecal Administration in a Horse. Fenbendazole was absorbed following intracecal administration in the horse indicating absorption processes in the hind gut. Concentrations of the parent molecule (expressed as AUC and C_max) were as high following intracecal administration as oral administration, although neither of the more oxidized metabolites achieved such good apparent bioavailability following intracecal administration (Table 3). Oxfendazole was also absorbed from the cecum, but its bioavailability even as the parent molecule was substantially lower than when administered orally.

The Effect of Piperonyl-butoxide on the Metabolism and Chirality of Benzimidazoles in Microsomes from Equine Liver. Oxfendazole was incubated in liver microsome preparations alone or with piperonyl-butoxide. Following a 1 h incubation period, significantly (P < 0.001) more FBZSO remained in the incubation mixture when the incubation was carried out in the presence of piperonyl-butoxide (data not shown). This was associated with a concurrent significant (P < 0.001) reduction in the generation of the sulfone (FBZSO_2) metabolite when the substrate was incubated with piperonyl-butoxide (Fig. 11).

Piperonyl-butoxide coinubcation also significantly reduced the metabolism of FBZ in microsome preparations and significantly (P < 0.001) reduced the generation of FBZSO in the 2.5 μM incubation only (data not shown). The generation of the sulfone metabolite was reduced at all incubation concentrations (Fig. 12); however, the generation of OH-FBZ was not affected by piperonyl-butoxide (data not shown).

The enantioselective metabolism of FBZSO was determined following incubations of FBZSO substrate as a racemate with and without piperonyl-butoxide. Microsomal metabolism was apparently enantioselective since the FBZSO-1 and FBZSO-2 enantiomers were present in the incubation medium after the incubation period in a ratio of >4:1 when FBZSO was incubated alone (Fig. 13). There was a marked change in the ratio when racemic FBZSO was incubated with piperonyl-butoxide such that the ratio of FBZSO-1/FBZSO-2 approached 1:1 (Fig. 14). Fenbendazole metabolism to the sulfoxide (FBZSO) was also shown to be enantioselective since FBZSO-1 predominated in reaction mixtures following incubation of FBZ. Piperonyl-butoxide affected the enantioselective character of the metabolism since the ratio of FBZSO-1/FBZSO-2 changed from 11 to 15:1 when FBZ was incubated alone to 3 to 6:1 when FBZ was incubated with piperonyl-butoxide (data not shown).

Discussion
The oral pharmacokinetic and excretion study confirms and extends the work of Marriner and Bogan (1985) who demonstrated that in the horse the bioavailability and residence time of the tested benzimidazoles (and metabolites) were lower and shorter, respectively than in ruminants (Marriner and Bogan, 1981a,b). The relatively low plasma concentrations of active moieties (FBZ, FBZSO) following administration of fenbendazole probably accounts for the increased dosage required to treat migrating larval and tissue stages of large strongyles and encysted mucosal stages of the cyathostomes (MAFF, 1983). It is also apparent that the sulfone metabolite predominates in plasma following administration of either the sulfoxide (FBZSO) or the sulfide (FBZ), and since this is known to confer relatively little anthelmintic activity, this could contribute to its relatively poorer efficacy. It is likely that the horse metabolizes sulfide and sulfone benzimidazoles to their sulfone metabolites more quickly than ruminants since the large AUC of the sulfone in the horse is not associated with a substantially increased MRT. In goats FBZSO had a MRT of 34.4 ± 1.52 h following administration of fenbendazole at 7.5 mg/kg (Benchaoi and McKellar, 1996), whereas in the present study, the MRT of the sulfone was 16.50 ± 1.00 h following fenbendazole administered at 10 mg/kg in the horse. The plasma bioavailability of oxfendazole was much greater than that of fenbendazole when each were administered orally at 10 mg/kg in the present study. This probably reflects better absorption of the parent molecule since the same metabolites are produced by each compound, and these would be expected to have similar metabolic and excretory rates. It is of interest that the sulfide moiety achieved 2.3 times greater concentrations (expressed as AUC) in horses administered FBZSO than FBZ as the parent molecule. It seems likely that the greater solubility of FBZSO than FBZ (3.01 and 0.07 mg/l, respectively, in buffer at pH 6.0 and 37°; Marriner and Bogan, 1985) permits its absorption at a rate exceeding its oxidative clearance and thus provide sufficient substrate for reductive metabolism to the sulfide. There is a paucity of information on the reductive mechanisms for benzimidazoles (Galtier, 1991), although it is clear from the present study that FBZSO is reduced to FBZ in the horse following oral and intravenous administration of FBZSO. This is most likely by hepatic processes, although secretion to and reduction in the gut with subsequent reabsorption of reduced moieties is also possible. Following administration of the sulfide as parent, its limited rate of absorption may be matched by rapid oxidative metabolism with consequent low concentrations achieved in plasma. The 12 h delay in appearance of benzimidazoles in feces following administration and the times to maximal concentration (24 h) and until no drug could be detected (72h) reflect the gut transit time of the horse, which has been shown to vary from 25.9 ± 4.5 h (Wolter et al., 1974) to 37.9 ± 5.3 h (Vander Noot et al., 1967).

The intravenous administration of FBZSO generated an atypical plasma concentration time curve. In the present study, FBZSO was dissolved in DMSO for administration, and for practical administration purposes, a very concentrated solution was prepared (500 mg/ml FBZSO) such that the volumes for delivery were 2 ml/100 kg (10 mg/kg). It is possible that upon delivery FBZSO came out of solution and deposited as a reservoir which, released over time, could have accounted for the increases in plasma concentration. This hypothesis is supported by recent work carried out in lambs, in which adminis-
tration of FBZSO in DMSO as 8 and 16% solutions generated atypical monoexponential plasma decline curves whereas a 4% solution generated a typical biexponential curve, and parallel in vitro dissolution experiments, in which recovery from plasma spiked at the same overall w/v rate with 8 and 16% FBZSO preparations was proportionately less than with a 4% solution (Sanchez et al., 2000). It is possible that extravasation during delivery could have affected the absorption pattern, although great care was taken to avoid this, and there was no evidence of it at the time of delivery. When FBZSO was coadministered with piperonyl-butoxide, the parent FBZSO and the FBZ metabolite achieved significantly (P < 0.01) greater concentrations (AUC and Cmax) in plasma, and this was apparently associated with inhibited metabolism of the FBZSO to FBZSO₂ since the elimination rates of each moiety appeared to be the same or faster (Figs. 6–8) when FBZSO was administered with piperonyl-butoxide, and because the drug was administered intravenously, absorption factors were precluded. The use of DMSO as the solvent for delivery of FBZSO could also have affected its metabolism. The reduction product of DMSO, dimethylsulphide is a known substrate for flavin-containing monooxygenase (FMO), and since FMO is responsible for the sulfoxidation of FBZ to FBZSO and for the sulforeduction of FBZSO to FBZ, the presence of an alternate substrate could affect these processes. Dimethyl sulfoxide is also known to inhibit several cytochrome P450 subtypes (2C9, 2C19, 2E1, and 3A4) in a concentration dependent manner (Hickman et al., 1998; Easterbrook et al.,

### TABLE 2

<table>
<thead>
<tr>
<th>Pharmacokinetic Parameters</th>
<th>Oxfendazole Administered Alone</th>
<th>Oxfendazole Administered with Piperonyl-butoxide</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FBZSO</td>
<td>FBZ</td>
</tr>
<tr>
<td>Cmax (μg/ml)</td>
<td>1.72 ± 0.26</td>
<td>0.06 ± 0.01</td>
</tr>
<tr>
<td>Tmax (h)</td>
<td>2.35 ± 1.28</td>
<td>14.56 ± 3.85</td>
</tr>
<tr>
<td>AUC₂₅₀ (μg · h/ml)</td>
<td>33.97 ± 3.29</td>
<td>1.03 ± 0.28</td>
</tr>
<tr>
<td>AUMC₂₅₀ (μg · h²/ml)</td>
<td>787.72 ± 161.91</td>
<td>21.38 ± 8.33</td>
</tr>
<tr>
<td>MRT₂₅₀ (h)</td>
<td>22.48 ± 2.43</td>
<td>18.55 ± 2.03</td>
</tr>
</tbody>
</table>

**Fig. 9.** Mean (±S.E.M.) plasma concentrations (μg/ml) of FBZSO-1 and FBZSO-2 in ponies following i.v. administration of oxfendazole (10 mg/kg) either alone (A) or in combination (B) with PB (31 mg/kg).

**Fig. 10.** Ratio of the percentage of FBZSO-1 and FBZSO-2 (μg/ml) in ponies following i.v. administration of oxfendazole (10 mg/kg) either alone (A) or in combination with piperonyl-butoxide (31 mg/kg) (B).

PB, piperonyl-butoxide.
While these confounding factors could not explain the early increase in concentration of FBZSO following intravenous administration, they could have contributed to reduced metabolism of benzimidazole moieties in both intravenous treatment groups (FBZSO alone and FBZSO with piperonyl-butoxide). The ratio of AUC-FBZSO/AUC-FBZSO2 was 1:2.98 in the horses given FBZSO orally in a preparation that did not contain DMSO and 1:1.38 following intravenous administration in DMSO, and although other factors such as concentration-dependent metabolism could have had an effect, this observation supports the hypothesis that DMSO affected the metabolism of FBZSO. Since DMSO was administered intravenously at the same dosage in horses given FBZSO alone and in those given FBZSO with piperonyl-butoxide, the differences in kinetics between the groups are not associated with DMSO. It is likely that the major effect of piperonyl-butoxide on FBZSO pharmacokinetics was associated with inhibited metabolism, and this is supported by liver microsomal studies described below. Nevertheless it is also possible that piperonyl-butoxide could have effects on blood flow and tissue perfusion and that the pharmacokinetic observations are the result of several pharmacological and physiological interactions.

Oxfendazole displayed enantioselective pharmacokinetics since the FBZSO-1 enantiomer predominated following administration of the racemate, and the FBZSO-1/FBZSO-2 ratio was 60:40 throughout most of the disposition period. The coadministration of piperonyl-butoxide dramatically altered the enantioselective pharmacokinetics of FBZSO since the FBZSO-2 enantiomer predominated for the first 12 h following administration after which the ratio changed in favor of FBZSO-1. The administration of DMSO could have affected the

### TABLE 3

The AUC and C\textsubscript{max} in a single horse following oral and intracecal administration of fenbendazole and oxfendazole at a dose rate of 10 mg/kg.

<table>
<thead>
<tr>
<th></th>
<th>Oral</th>
<th>Intracecal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AUC</td>
<td>C\textsubscript{max}</td>
</tr>
<tr>
<td>Fenbendazole</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FBZ</td>
<td>0.33</td>
<td>0.015</td>
</tr>
<tr>
<td>FBZSO</td>
<td>0.89</td>
<td>0.019</td>
</tr>
<tr>
<td>FBZSO2</td>
<td>2.18</td>
<td>0.041</td>
</tr>
<tr>
<td>Oxfendazole</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FBZ</td>
<td>0.73</td>
<td>0.036</td>
</tr>
<tr>
<td>FBZSO</td>
<td>3.69</td>
<td>0.171</td>
</tr>
<tr>
<td>FBZSO2</td>
<td>7.11</td>
<td>0.288</td>
</tr>
</tbody>
</table>
absolute proportions of enantiomers in the present study since inhibition of FMO oxidation/reduction by DMSO/dimethylsulphide could have reduced oxidation of FBZ or caused an accumulation of FBZSO thus enhancing its stereoselective reduction. Since DMSO was given to both groups of animals (with and without piperonyl-butoxide), the enantioselective changes in pharmacokinetics can be attributed to the piperonyl-butoxide.

The metabolism of sulfide to sulfoxide benzimidazoles is thought to be principally catalyzed by the FMO system (Galtier et al., 1986) whereas metabolism of sulfoxide to sulfone is thought to be catalyzed by hepatic cytochrome P450 (Souhaili-El Amri et al., 1988). It has also been demonstrated that the FMO system is stereo-selective in favor of the (+) sulfoxide of the related benzimidazole albendazole whereas cytochrome P450 systems specifically use (−) albendazole sulfoxide as substrate (Morani et al., 1995).

In the present study piperonyl-butoxide coadministration dramatically altered the generation of FBZSO enantiomers in favor of FBZSO-2, suggesting that the cytochrome P450 systems on which it acts may be responsible for FBZSO-2 metabolism. The eudismic (potency) ratio of the benzimidazole sulfoxides is unknown, however, the alterations in enantiomer generation together with the alteration in achiral metabolism of benzimidazoles by piperonyl-butoxide could have a major impact on the efficacy of benzimidazole sulfides and sulfoxides in the horse.

The small number of animals (n = 6) and large interanimal variation in results meant that no significant differences could be detected for pharmacokinetic parameters of the separate drug moieties following oral administration of fenbendazole with or without food. Nevertheless when the AUC values of active moieties (FBZSO and FBZ) were summed, they achieved almost four times the concentration in unfed (2.19 µg · h/ml) than fed (0.59 µg · h/ml) horses, and it would seem appropriate that for systemic parasitic infections, fenbendazole be given following food restriction.

It is known that solutes move through the gut more rapidly than particles and that small particles move more rapidly than large ones (Warner, 1981). Benzimidazoles are administered orally as suspensions, and their particulate size relative to gut transport is more likely to be associated with the size of concurrent food particles to which they become adsorbed. Consequently, it would be expected that transit would be faster on an empty stomach and thus systemic absorption reduced. However, it is known that in the hind gut of the rabbit and some other animals, solutes and small particles are selectively retained (Warner, 1981) and both FBZ and FBZSO were extensively absorbed when administered directly into the cecum of the horse (see Table 3). The anatomy of the equine ileo-cecal valve makes retrograde delivery of the drug into the distal ileum from the cecum unlikely, although not impossible.

The coadministration of piperonyl-butoxide p.o. at 63 mg/kg increased the AUC of the active moieties (FBZSO + FBZ) of FBZ administered at 10 mg/kg in fed ponies by 13 times, and this may represent a strategy for improving the efficacy of benzimidazoles in the horse. Benchaoui and McKellar (1996) have shown that piperonyl-butoxide coadministration greatly improves the activity of FBZ against nematodes resistant to benzimidazoles in sheep when administered at normal therapeutic doses. They have also demonstrated in a dose titration study that a dose of 31 mg/kg piperonyl-butoxide significantly improved the bioavailability of benzimidazoles in sheep, and given the very significant increases in bioavailability demonstrated in the present study when piperonyl-butoxide was administered at 31 mg/kg (intravenous experiment) and 63 mg/kg (oral experiment), it is likely that it will prove effective at lower dosages. It is of interest that in the present oral administration study, piperonyl-butoxide produced a greater effect on benzimidazole bioavailability when it was given with food (Table 4), and since this effect is the opposite of that described above when the benzimidazole was given with food in the absence of piperonyl-butoxide, it is possible that the food decreased the absorption of the piperonyl-butoxide, thus improving its dynamic effects on liver metabolism. Piperonyl-butoxide concentrations were not measured in the present study.

The equine liver microsomal studies support much of the in vivo work described above. Piperonyl-butoxide significantly inhibited the sulfonation of FBZSO and the sulfoxidation and sulfonation of FBZ. It was also apparent that FBZSO-2 was metabolized more rapidly than FBZSO-1 in equine liver microsomes and that piperonyl-butoxide altered the metabolism such that the ratio of FBZSO-1/FBZSO-2 remaining after incubation was much closer to unity. The eudismic ratio of FBZSO is unknown, but it is apparent that the effects of piperonyl-butoxide on efficacy of benzimidazoles could be due to changes in enantiomer ratio as well as absolute increases in active moiety in plasma. In the horse the relationship between gastrointestinal concentrations, plasma concentrations, and efficacy of benzimidazoles has not been clearly defined. In ruminants it is thought that redistribution from plasma into gut is responsible for much of the activity of the drug and that

### Table 4

<table>
<thead>
<tr>
<th></th>
<th>FBZSO</th>
<th>FBZ</th>
<th>FBZSO/2</th>
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</thead>
<tbody>
<tr>
<td>+ Food</td>
<td>0.01 ± 0.00</td>
<td>0.04 ± 0.01</td>
<td>0.09 ± 0.02</td>
</tr>
<tr>
<td>− Food</td>
<td>0.03 ± 0.01</td>
<td>0.02 ± 0.01</td>
<td>0.04 ± 0.02</td>
</tr>
<tr>
<td>+ Food + PB</td>
<td>0.13 ± 0.07</td>
<td>0.37 ± 0.10</td>
<td>0.11 ± 0.03</td>
</tr>
<tr>
<td>− Food + PB</td>
<td>0.04 ± 0.00</td>
<td>0.16 ± 0.02</td>
<td>0.05 ± 0.01</td>
</tr>
<tr>
<td>+ Food</td>
<td>0.15 ± 0.06</td>
<td>0.44 ± 0.01</td>
<td>1.47 ± 0.19</td>
</tr>
<tr>
<td>− Food</td>
<td>1.87 ± 0.60</td>
<td>0.32 ± 0.11</td>
<td>1.20 ± 0.49</td>
</tr>
<tr>
<td>+ Food + PB</td>
<td>2.08 ± 0.99</td>
<td>5.81 ± 1.96</td>
<td>1.44 ± 0.33</td>
</tr>
<tr>
<td>− Food + PB</td>
<td>0.82 ± 0.22</td>
<td>3.51 ± 0.40</td>
<td>1.29 ± 0.27</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th></th>
<th>AUC (µg · h/ml)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>+ Food</td>
<td>7.53 ± 1.28</td>
<td>11.40 ± 1.63</td>
<td>18.77 ± 1.70</td>
</tr>
<tr>
<td>− Food</td>
<td>22.94 ± 7.35</td>
<td>11.68 ± 3.29</td>
<td>30.86 ± 6.00</td>
</tr>
<tr>
<td>+ Food + PB</td>
<td>13.56 ± 2.44</td>
<td>12.63 ± 1.97</td>
<td>20.19 ± 1.72</td>
</tr>
<tr>
<td>− Food + PB</td>
<td>21.05 ± 2.53</td>
<td>17.51 ± 1.45</td>
<td>28.10 ± 7.10</td>
</tr>
</tbody>
</table>
bulk flow through the gastrointestinal tract is less important (Baggot and McKellar, 1994).

References


Ogbourne CP and Duncan JL (1977) *Strongylus vulgaris* in the horse: its biology and veterinary importance, in *CHI Miscellaneous Publications No. 4*, pp 40, Commonwealth Agricultural Bureau, Farnham Royal, UK.


