Plasma achiral and chiral pharmacokinetic behaviour of intravenous oxfendazole co-administered with piperonyl butoxide in sheep

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Sánchez, S., Small, J., Jones, D. G., McKellar, Q. A. Plasma achiral and chiral pharmacokinetic behaviour of intravenous oxfendazole co-administered with piperonyl butoxide in sheep. *J. vet. Pharmacol. Therap.* **25,** 7–13.

Co-administration of piperonyl butoxide (PB) potentiates fenbendazole (FBZ) in small ruminants. The resultant increase in bioavailability of FBZ and its metabolite oxfendazole (OFZ) has important implications for the efficacy of these drugs against benzimidazole (BZD)-resistant strains of *Teladorsagia circumcincta.* This study evaluated the racemic (achiral) and enantiomeric (chiral) plasma disposition kinetics of OFZ and its metabolites after the co-administration of PB and OFZ in sheep. Six 6–8-month-old, parasite-free, female Dorset sheep (30–40 kg) were used in a two-phase crossover experiment. In phase I, three sheep received 30 mg/kg PB orally, followed by a single intravenous (i.v.) injection of OFZ at 5 mg/kg. The other three animals were treated similarly except that 5 mL of water replaced PB. In phase 2, treatments for the two groups were reversed and were given 14 days after the initiation of phase I. Three analytes OFZ, FBZ and fenbendazole sulphone ($FBZSO_2$) were recovered in plasma up to 48 h post-treatment in both experimental groups. Achiral and chiral pharmacokinetic (PK) profiles for OFZ, after the co-administration of PB, were characterized by a significantly greater area under the concentration–time curve (*AUC*) and a longer mean residence time (*MRT*). Chiral OFZ distribution ratios were comparable in both treatment groups. Piperonyl butoxide treatment markedly influenced the plasma PK profiles for FBZ and $FBZSO_2$ following OFZ administration. Production of FBZ was enhanced as reflected by increased (> 60%) *AUC*, delayed T_{max} and a significantly delayed (> 45%) elimination ($t½_{el}$). Although *AUC* values for $FBZSO_2$ were not significantly different between groups, this metabolite was depleted more slowly from plasma ($t½_{el}$ > 60% and *MRT* > 42%) following PB treatment. This study demonstrated that PB co-administration is associated with an inhibition of OFZ biotransformation, as evidenced by the significantly higher plasma concentrations of OFZ and FBZ, and this could have important implications in terms of antiparasite therapy against BZD-resistant parasite strains.

(Paper received 10 March 2001; accepted for publication 16 August 2001)

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INTRODUCTION

Several anthelmintic compounds with different activity spectra and pharmacological properties are currently available on the veterinary market. However, intensive use over many years has led to increased occurrence of parasite resistance, and consequently reduced the efficacy of these drugs. Despite extensive research by pharmaceutical companies, no major new compounds have emerged since the avermectins became available in the 1980s. New pharmacological strategies are, therefore, needed to improve the performance of existing anthelmintics. Characterization of pharmacokinetic (PK) interactions offers an alternative approach for optimizing the use and preserving the efficacy of available drugs, by increasing and targeting drug concentrations to specific tissue sites.

Benzimidazoles (BZD) and pro-BZD are extensively metabolized in the host and their metabolic pattern and PK behaviour are pivotal to the attainment of sustained concentrations of active parent drug and/or metabolites at sites of parasitic infestation (Lanusse & Prichard, 1993). For example, fenbendazole (FBZ), the parent drug, is oxidised in the liver to oxfendazole (OFZ), the first pharmacologically active metabolite. Oxfendazole then

undergoes sulphonation, involving the cytochrome P-450 complex, to form fenbendazole sulphone ($FBZSO_2$) which is inactive (McKellar & Scott, 1990; Lanusse & Prichard, 1993). In a more recent study, Benchaoui and McKellar (1996) demonstrated that co-administration of piperonyl butoxide (PB) potentiates FBZ in sheep and goats, and the consequent increased bioavailability of FBZ and achiral OFZ reported had implications for the efficacy of these drugs against BZD-resistant strains of *Teladorsagia circumcincta*. However, further studies to define precisely how PB potentiates FBZ and its metabolites are required. The aim of this study was to assess the achiral and chiral plasma disposition kinetics of OFZ (given intravenously) and its metabolites after the oral co-administration of PB in sheep.

MATERIALS AND METHODS

Experimental design

Six parasite-free, 6–8-month-old, female Dorset sheep (30–40 kg) were used in a two phase crossover experiment as follows. In Phase I, three sheep (Group 1) were given 30 mg/kg PB orally, via a stomach tube, followed 30 min later by a single i.v. injection of OFZ (50:50% racemate, 4% in dimethyl sulphoxide) at 5 mg/kg liveweight. Group 2 was treated similarly except that 5 mL of water was used as a placebo in place of PB. In Phase II, treatments for the two groups were reversed and administered 14 days after the initiation of Phase I. Venous whole blood samples were taken by jugular venepuncture using 10 mL heparinized Vacutainers® (Becton Dickinson UK Ltd, Oxfordshire, UK). Sampling times were at 0 (blank sample) and at 0.086, 0.16, 0.25, 0.5, 0.75, 1, 2, 3, 4, 5, 6, 8, 10, 12, 18, 24, 30, 36 and 48 h post-treatment. All blood samples were centrifuged at $3000 \times g$ for 10 min to separate plasma. Heparinized plasma samples were frozen at –20°C and analysed by high-performance liquid chromatography (HPLC). All sheep were fed 1 h before treatment and had free access to water. All animal procedures and management protocols were approved by the Moredun Research Institute Experiments and Ethics Committee and carried out under approved British Home Office licences in accordance with the Animals (Scientific Procedures) Act, 1986.

Analytical procedures

Extraction of OFZ and its metabolites from plasma. Oxfendazole, FBZ, $FBZSO_2$ and oxibendazole (OXB) (internal standard) were isolated from plasma by solid phase extraction using C_{18} Bond Elute® cartridges (Varian UK Ltd, Surrey, UK). Fifteen microlitres of OXB (50 μg/mL) authentic standard were added to 750 μL plasma in a glass test tube. Five hundred microlitres of this mixture were applied to the C_{18} cartridge (preconditioned with 1 mL of methanol followed by 1 mL HPLC-grade water) in a vacuum system (Vac-Elut®, Varian UK Ltd, Surrey, UK). Samples were washed with 1 mL of HPLC-grade water, eluted with 2 mL of HPLC methanol, concentrated to dryness in a vacuum concentrator (Speed-Vac®, Thermo Savant, Holbrook, NY, USA), and then reconstituted with 200 μL of mobile phase.

Achiral analysis of OFZ and its metabolites. Reconstituted plasma extracts (50 μL) were analysed on a Waters® 625 modular HPLC system (Waters UK Ltd, Herts, UK) with ultraviolet (UV) detection (Waters® 486) at 296 nm. Separation was based on a modified linear gradient previously reported by Benchaoui and McKellar (1996). A mobile phase comprising 35:65 acetonitrile and water (in 0.5% of acetic acid) was used to elute the analytes from the stationary phase (5 μm, 150 mm × 4.6 mm, C_{18} column, Nemesis®, Phenomenex, Cheshire, UK). The linear gradient was constructed as follows:

Time (min)	Flow (mL/min)	Acetonitrile (%)	B (water + acetic acid 0.5%) (%)
0.00	0.80	35	65
4.00	0.80	60	40
4.10	1.00	60	40
9.00	1.00	60	40
10.0	0.80	35	65
12.0	0.80	35	65

Authentic standards (FBZ, batch# D383; OFZ, batch# A 011; $FBZSO_2$ batch# MR 12972) for the method validation were obtained from Hoechst, AG, Frankfurt, Germany. The internal standard (OXB) was purchased from Sigma UK Ltd, Dorset, UK (Cat No: O3132). Retention times of the authentic standards were 3.14 min (OXB), 3.75 min (OFZ), 5.60 min ($FBZSO_2$) and 7.50 min (FBZ).

Precision (intra- and inter-assay) was determined by analysing replicates of plasma fortified ($n = 5$) with each compound at three different concentrations (0.25, 0.5 and 1 μg/mL). Coefficients of variation ranged from 1.2 to 8.5%, and the mean relative recoveries for FBZ, OFZ and $FBZSO_2$ were 95.6% (± 3.64), 91.4% (± 2.06) and 97.6% (± 5.05), respectively.

Calibration curves for each analyte, constructed by least squares linear regression analysis, showed good linearity, with Pearson's correlation rate of elimination between 0.987 and 0.988. The lack of fit test was used to corroborate the method linearity. The limit of quantification (LOQ) was calculated as 10 times of the standard deviation (SD) of the limit of detection (LOD). The latter was estimated from the baseline noise at the retention times of each analyte of interest. Limit of quantifications obtained by this method were 0.025, 0.112 and 0.035 μg/mL for FBZ, OFZ and $FBZSO_2$, respectively. Experimental values below the LOQ were not included in the PK analysis.

Chiral analysis of OFZ. Percentages of the peak areas of OFZ enantiomers [(–) OFZ1 and (+) OFZ2] were estimated using an α-glycoprotein analytical column (Chrom-Tech®, Cheshire, UK)

in an isocratic HPLC system (Waters® 625) using water/acetonitrile, 93:7, as mobile phase. (–) OFZ1 and (+) OFZ2 were detected by UV Waters® (486) at 296 nm. The physicochemical extraction of the chiral analytes was identical to that detailed above for the achiral analysis, except that concentrated extracts were reconstituted in water/acetonitrile (96.5:3.5). The relative retention times were 6.08 and 7.80 min for the (–) OFZ1 and (+) OFZ2 enantiomers, respectively.

Pharmacokinetic analysis

Pharmacokinetic analysis of plasma concentration vs. time data for OFZ, FBZ and $FBZSO_2$ was performed using the computer software package PK Solution® 2.0 (Summit Research Services, Ashland, OH, USA). The biexponential concentration vs. time curves for OFZ and metabolites in plasma were best-fitted by a biexponential curve (OFZ: $r^2 = \sim 0.98$, $FBZSO_2$ and FBZ $r^2 = 0.98$–0.99) supported by the following equations (Notari, 1987):

$$\mathrm{Cp} = A\mathrm{e}^{-\alpha t} + B\mathrm{e}^{-\beta t(\mathrm{OFZ})}$$

$$\mathrm{Cp} = B\mathrm{e}^{\beta t} + B\mathrm{e}^{-Kt(\mathrm{FBZSO_2\ and\ FBZ})}$$

The distribution and elimination half-lives of OFZ and its metabolites after i.v. OFZ administration were calculated as

$\ln 2/\alpha$ (distribution phase$^{[\mathrm{OFZ}]}$ or metabolite formation phase$^{[\mathrm{FBZSO_2\ and\ FBZ}]}$)

$\ln 2/\beta$ (elimination phase)

The sum of rate of eliminations A and B was applied to estimate the plasma concentration of OFZ after i.v. administration. The total body clearance of the OFZ in plasma (Cl_B) was calculated using the conventional equation described by Gibaldi and Perrier (1982): $Cl_B = \mathrm{Dose}/AUC$. The volume of distribution (Vd_{area}) of the central compartment was estimated by the following equation:

$$Vd_{area} = \mathrm{Dose}/AUC \times \beta$$

The maximum concentrations (C_{max}) and time to C_{max} (T_{max}) for $FBZSO_2$ and FBZ obtained in plasma following i.v. administration of OFZ were extrapolated from the plotted concentration–time-curve of each animal. Trapezoidal *AUC*s were calculated by the following equation again according to Gibaldi and Perrier (1982):

$$AUC_{(0-t)} = \sum_{i=0}^{n-1} t_{i+1} - t_i/2 \times (C_i + C_{i+1})$$

Mean residence times (*MRT*) were calculated as $MRT = AUMC/AUC$ where *AUC* is the area under the concentration vs. time curve from 0 to infinity and *AUMC* is the area under the curve of the product of time and the plasma drug concentration vs. time from 0 to infinity.

Statistical analysis

Mean plasma PK variables for OFZ obtained for both groups were statistically compared by nonparametric analysis, using the Mann–Whitney test. Means were considered significantly different at $P < 0.05$. Pharmacokinetic variables are reported as geometrical mean ± SD. Elimination half-lives for OFZ and metabolites were calculated as harmonic mean.

RESULTS

Three analytes, namely OFZ, FBZ and $FBZSO_2$, were recovered in plasma for up to 48 h after treatment in both experimental groups. The achiral PK profile of OFZ, after the co-administration with PB, was characterized by a significantly ($P < 0.05$) greater *AUC* and a longer *MRT* (Table 1; Fig. 1). Plasma PK profiles for FBZ (generated from OFZ) and $FBZSO_2$ were also influenced by PB pretreatment (Figs 2 & 3; Table 2). Enhanced production of FBZ was reflected in greater *AUC* values, delayed T_{max} and a significantly ($P < 0.05$) sustained elimination rate ($t½_{el}$) following OFZ + PB treatment (Fig. 2; Table 2). However, *AUC* values for $FBZSO_2$ showed no statistical differences between groups, although this metabolite was depleted more slowly from plasma in the PB-treated group (Fig. 3; Table 2). Figure 4a,b show the chiral OFZ PK profiles after both experimental treatments. Depletion of the OFZ enantiomers was comparatively higher in the PB-treated group but the percentages of (–) OFZ1 and (+) OFZ2 were unaffected by PB treatment (Table 3).

Table 1. Pharmacokinetic (PK) parameters (mean ± SD, $n = 6$) for oxfendazole (OFZ), in sheep, after the intravenous (i.v.) dose of a racemic mixture of OFZ (5 mg/kg liveweight) either alone, or combined with piperonyl butoxide (PB; 30 mg/kg liveweight, orally)

	OFZ	
PK parameters	–PB	+PB
A (μg/mL)	2.55 ± 1.28	2.70 ± 1.27**
α (h^{-1})	0.38 ± 0.18	0.39 ± 0.34
$t½\alpha$ (h)	1.80 ± 0.58	1.75 ± 1.38
B (μg/mL)	4.62 ± 1.89	5.13 ± 0.36
β (h^{-1})	0.12 ± 0.00	0.08 ± 0.01
$t½\beta$ (h)	6.01 ± 0.44	7.87 ± 1.09***
AUC (μg·h/mL)	48.9 ± 10.6	66.6 ± 16.0*
AUMC (μg·h^2/mL)	377 ± 96.0*	705 ± 267***
MRT (h)	7.89 ± 0.90	11.8 ± 4.16***
Vd_c (L/kg)	647 ± 140	549 ± 0.283
Vd_{area} (mL/kg)	887 ± 313	860 ± 108
Vd_{ss} (mL/kg)	786 ± 182	793 ± 130
Cl_B (mL·kg/h)	102 ± 27.1	75.1 ± 16.0**

A, B: *y*-axis intercepts for the phases of the absorption and elimination, respectively; α, β: rate constant for the absorption and elimination, respectively; $t½$: half-life; *AUC*: area under the concentration–time curve; *AUMC*: area under first moment curve; *MRT*: mean residence time; Vd_c: apparent volume of distribution of the central compartment; Vd_{area}: apparent volume of distribution (area method); Vd_{ss}: apparent volume of distribution at steady-state; Cl_B: total body clearance.

Values are statistically different from the control group (–PB) at $^*P < 0.05$, $^{**}P < 0.01$ or $^{***}P < 0.005$ by Mann–Whitney test.

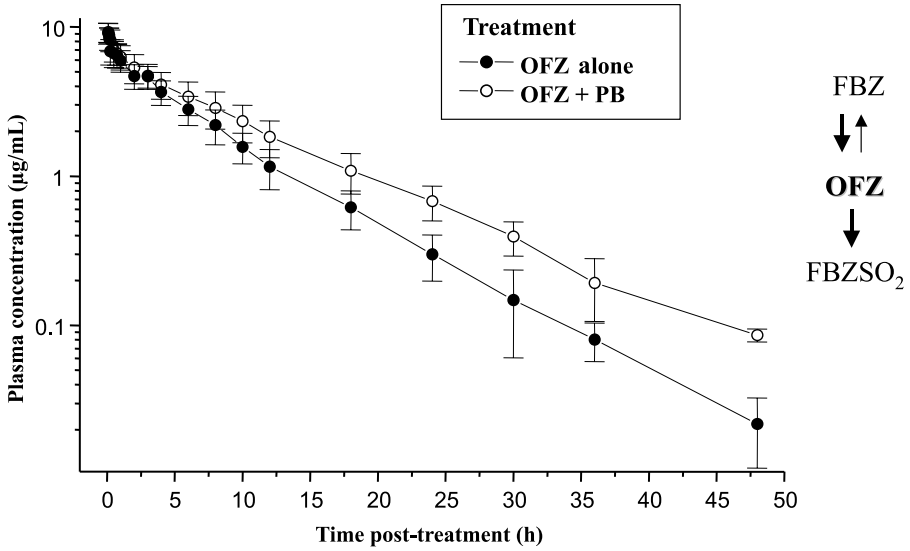

Fig. 1. Comparative plasma concentrations of oxfendazole (OFZ) in sheep ($n = 6$) given a racemic mixture of OFZ (5 mg/kg liveweight, intravenously) either alone or co-administered with piperonyl butoxide (PB; 30 mg/kg liveweight, given orally 30 min before OFZ administration).

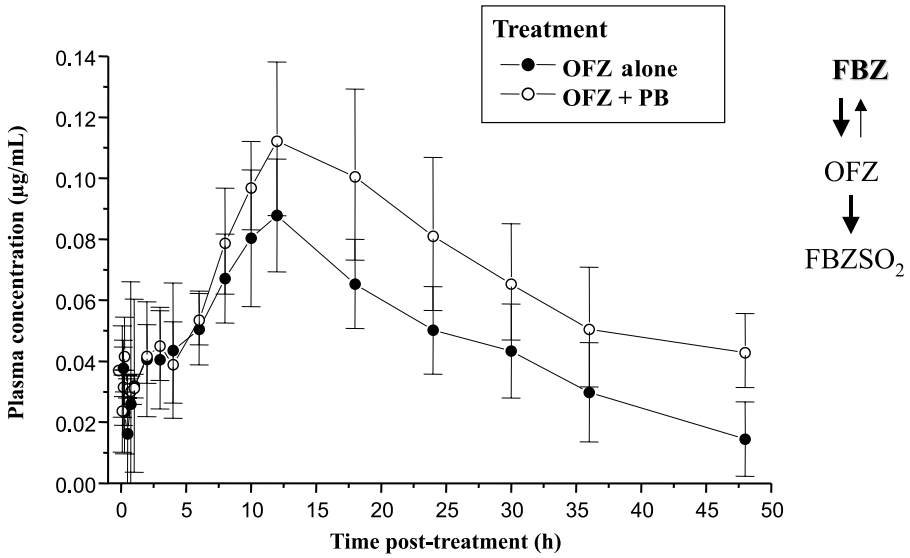

Fig. 2. Comparative plasma concentrations of fenbendazole (FBZ) in sheep ($n = 6$) given oxfendazole (OFZ; 5 mg/kg liveweight, intravenously) either alone or co-administered with piperonyl butoxide (PB; 30 mg/kg liveweight, given orally 30 min before OFZ administration).

DISCUSSION

A variety of new management and pharmacological strategies have been developed within the last decade designed to optimize the use of antiparasitic drugs in order to improve efficacy against an emerging background of resistant parasite strains (McKellar, 1994). Methods which facilitate improved uptake and persistence of anthelmintics in the host, particularly at local sites of infestation, have been sought and one approach has been to utilize modulators of drug absorption and metabolism. For example, recent studies have demonstrated that biotransformation of BZD can be modified in the presence of methimazole in sheep and calves (Lanusse *et al.*, 1993, 1995) and PB in sheep and horses (Benchaoui & McKellar, 1996; Gokbulut *et al.*, 2000a,b). Methimazole is an inhibitor of the flavine mono-oxygenase (FMO) complex and PB interferes with cytochrome P450 complex-mediated reactions, both of which are involved in the metabolism of BZD. This study has extended these previous reports and has provided evidence that co-treatment with PB substantially improved the maintenance and persistence of the pharmacologically active metabolites (OFZ and FBZ), but was associated with decreased production of the inactive sulphone ($FBZSO_2$), in ovine plasma following i.v. administration of OFZ.

Oxfendazole is metabolized either irreversibly to $FBZSO_2$ via cytochrome P450-mediated sulphonation or reversibly, by sulphoxidation/reduction, to the parent sulphide (FBZ) in an FMO-complex-mediated reaction (Murray *et al.*, 1992). Achiral analysis of plasma concentrations showed that OFZ was depleted more slowly after PB co-administration, as evidenced by a significantly ($P < 0.005$) prolonged elimination half-life (7.99 ± 1.09) compared with animals given OFZ alone (6.03 ± 0.44; Table 1). Moreover, in PB-treated animals, plasma concentrations of OFZ were increased and this was reflected by significantly ($P < 0.05$) higher *AUC* values (68.0 ± 16.0) and longer *MRT* (12.30 ± 4.16) compared with the control group.

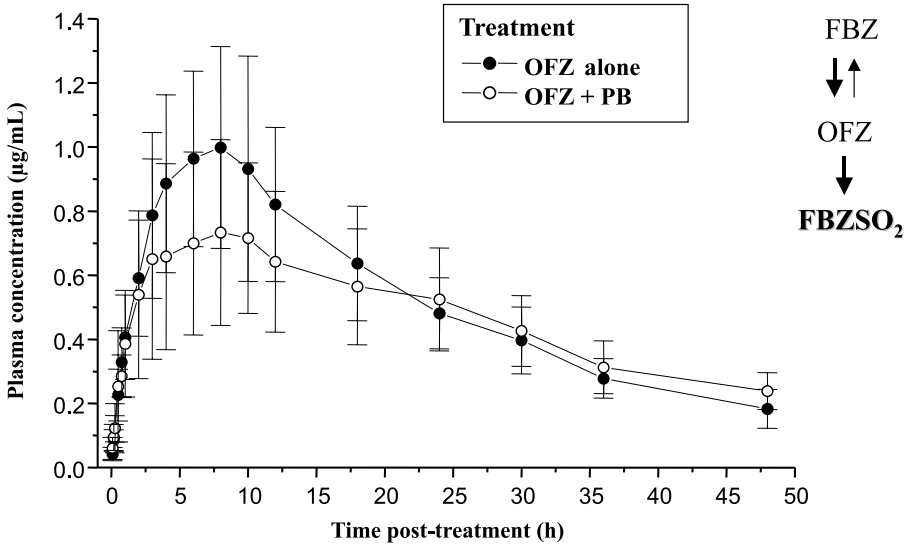

Fig. 3. Comparative plasma concentrations of fenbendazole sulphone ($FBZSO_2$) in sheep ($n = 6$) given oxfendazole (OFZ; 5 mg/kg liveweight, intravenously) either alone or co-administered with piperonyl butoxide (PB; 30 mg/kg, given orally 30 min before OFZ administration).

Table 2. Pharmacokinetic (PK) parameters (mean ± SD, $n = 6$) for fenbendazole (FBZ) and fenbendazole sulphone ($FBZSO_2$), in sheep, after the intravenous (i.v.) dose of a racemic mixture of OFZ (5 mg/kg liveweight) either alone, or combined with piperonyl butoxide (PB; 30 mg/kg liveweight, orally)

	FBZ		$FBZSO_2$	
PK parameters	−PB	+PB	−PB	+PB
C_{max} (µg/mL)	0.09 ± 0.02	0.11 ± 0.02	0.98 ± 0.33	0.72 ± 0.28
T_{max} (h)	9.28 ± 1.03	11.6 ± 0.89**	7.19 ± 0.63	7.91 ± 1.26
AUC (µg·h/mL)	1.95 ± 0.40	3.15 ± 0.70**	25.3 ± 6.10	22.0 ± 7.10
AUMC (µg·h^2/mL)	64.0 ± 9.00	171 ± 6.14*	733 ± 194	1095 ± 254*
$t½\beta$ (h)	15.8 ± 3.47	22.6 ± 6.14*	16.2 ± 1.63	23.3 ± 2.77***
MRT (h)	27.6 ± 4.81	40.6 ± 7.51*	26.1 ± 2.45	37.1 ± 4.54***

C_{max}: maximum plasma concentration; T_{max}: time at C_{max}; *AUC*: area under the concentration–time curve; *AUMC*: area under first moment curve; $t½\beta$: elimination half-life; *MRT*: mean residence time. Values statistically different from the control group (−PB) at $^*P < 0.05$, $^{**}P < 0.01$ or $^{***}P < 0.005$ by Mann–Whitney test.

There was also evidence from the time vs. concentration curve (Fig. 3) and PK analysis (Table 2), that the rate of $FBZSO_2$ production was decreased in PB-treated sheep. These results are consistent with the recognized properties of PB as an inhibitor of cytochrome P450.

In contrast, based on the same criteria (Fig. 2; Table 2), the production of FBZ was enhanced following PB treatment. Under normal conditions sulphoxidation of FBZ to OFZ predominates over the opposite sulphoreduction (i.e. OFZ to FBZ) in the reversible, FMO-complex-mediated reaction described earlier (Murray *et al.*, 1992; Lanusse *et al.*, 1993). The observed increase in FBZ accumulation may have been due to the additional OFZ available as substrate as a result of PB-inhibited sulphonation. Alternatively, PB may have had a more direct effect on the OFZ/FBZ biotransformation pathway. Evidence to support the latter comes from *in vitro* studies with rat (Benchaoui & McKellar, 1996) or equine (Gokbulut *et al.*, 2000b) liver microsomes in which PB was shown to inhibit both the sulphoxidation and sulphonation of FBZ. The possibility raised by Benchaoui and McKellar (1996) that PB could enhance drug absorption seems unlikely given the observed increases in plasma OFZ obtained after i.v. administration of the latter. The increased availability of both active moieties (OFZ and FBZ), following OFZ treatment in the presence of a metabolic inhibitor such as PB, could have important implications for efficacy particularly given that FBZ has been shown, at least *in vitro*, to possess a greater binding affinity for parasite tubulin than OFZ (Lacey *et al.*, 1987).

In contrast to the present studies and previous *in vitro* work, Gokbulut *et al.* (2000b) were unable to demonstrate significant decreases in sulphonation of achiral OFZ in ponies treated with PB. This may have been because of differences in dosage strategies or inherent species differences in BZD metabolism between sheep and horses.

The chirality of OFZ has been well characterized and both enantiomers (+) OFZ2 and (–) OFZ1, have been detected in the plasma of sheep treated with the pro-chiral sulphide, FBZ (Delatour *et al.*, 1990). However, the precise impact of its chirality and the enantioselective PK of OFZ on antiparasitic activity is unclear. It is known that the FMO-mediated sulphoxidation of FBZ favours production of (+) OFZ2, whilst cytochrome P450 sulphonation selectively uses (–) OFZ1 as substrate (Light *et al.*, 1982; Waxman *et al.*, 1982; Ziegler, 1989). In the present study, the (+) OFZ2 and (–) OFZ1

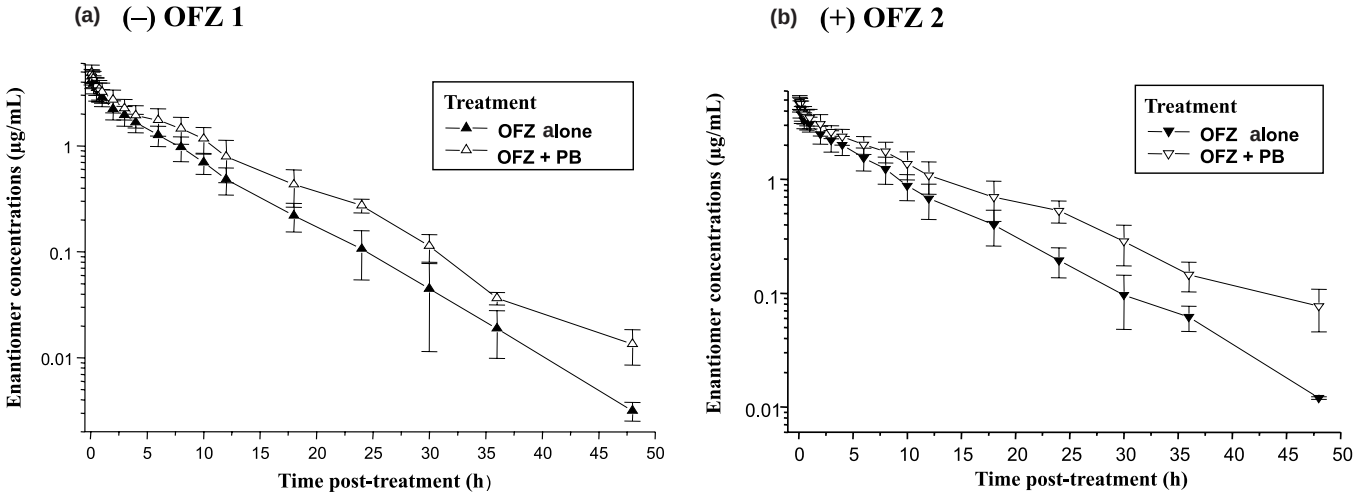

Fig. 4. Comparative plasma concentrations of oxfendazole (OFZ) enantiomers: (a) (–) OFZ1 and (b) (+) OFZ2 in sheep ($n = 6$) given OFZ (5 mg/kg liveweight, intravenously) either alone or co-administered with piperonyl butoxide (PB; 30 mg/kg liveweight, administered by oral route 30 min before OFZ injection).

Table 3. Area under the concentration–time curves (*AUC*) values obtained for racemic oxfendazole (OFZ) and its enantiomers (–) OFZ1 and (+) OFZ2, after the intravenous (i.v.) dose of a racemic mixture of OFZ (5 mg/kg liveweight) either alone, or combined with piperonyl butoxide (PB; 30 mg/kg liveweight, orally)

	AUC		
Treatment	OFZ racemate	(–) OFZ1	(+) OFZ2
OFZ alone	50.0 ± 10.6	22.0 ± 4.66 (44%)	28.0 ± 5.93 (54%)
OFZ + PB	68.0 ± 16.0	30.0 ± 7.04 (44%)	38.0 ± 8.64 (54%)

Percentages shown in brackets are the proportions of each enantiomer obtained after individual kinetic analysis.

components of the OFZ racemate administered were depleted in parallel irrespective of PB treatment, although the latter was associated with higher plasma concentrations of both enantiomers (Fig. 4a,b). This is consistent with the reported inhibitory effects of PB on both the sulphoxidation/reduction and sulphonation pathways (Benchaoui & McKellar, 1996; Gokbulut *et al.*, 2000b).

The proportionate evolution of (+) OFZ2 and (–) OFZ1 in plasma altered substantially with time but, again, was unaffected by PB treatment. Immediately after i.v. OFZ (0.086–0.25 h) the (+) OFZ2: (–) OFZ1 percentage ratio was 50:50 but, by 48 h post-treatment this had changed to 80:20 in favour of (+) OFZ2. As postulated by others (Delatour *et al.*, 1990), in previous studies on FBZ and albendazole metabolism in sheep, the present results may reflect the irreversible and selective sulphonation of the (–) OFZ1 enantiomer or differences in plasma protein binding resulting in different renal clearance rates for the two enantiomers.

The lack of effect of PB treatment on chiral OFZ distribution is confirmed by the PK data (Table 3) which indicated that there were no significant differences in the proportionality of the *AUC* values obtained for (+) OFZ2 and (–) OFZ1 in the two treatment groups. This differs from a recent work in horses in which significant differences in chiral distribution were reported for animals treated with i.v. OFZ either alone or in combination with PB (Gokbulut *et al.*, 2000b). The reasons for this are unclear but significant species differences in the evolution of albendazole sulphoxide enantiomer distribution in the plasma of humans, dogs and rats, following albendazole sulphide treatment, have been noted previously (Delatour *et al.*, 1991).

As conclusion, this trial demonstrated that PB co-administration is associated with an inhibition of OFZ biotransformation, as evidenced by the significantly higher plasma concentrations of OFZ and FBZ, and this could have relevant implications in terms of antiparasite therapy against BZD-resistant parasite strains.

ACKNOWLEDGMENTS

Part of this work was supported by the Scottish Executive Rural Affairs Department (SERAD).

Dr Sergio Sánchez gratefully acknowledges the financial support of the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET, Argentina) and SECyT, UNCPBA (Argentina). The authors also thank members of the MRI Clinical Division for their expert help with the animal work.

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