Effects of formulation concentration on intravenous pharmacokinetics, chirality and *in vitro* solubility of oxfendazole and its metabolites in sheep

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This study compared pharmacokinetic (PK) profiles in sheep dosed intravenously with three different concentrations of oxfendazole (OFZ). An *in vitro* plasma OFZ solubility study provided additional information on plasma saturation. For the PK study, 18 adult, parasite-free, female Suffolk cross sheep, allocated into three groups ($n = 6$), were treated intravenously, at a dose rate of 5 mg/kg bodyweight, with aqueous formulations containing at 4, 8 or 16% OFZ. Plasma drug concentrations were measured, for up to 72 h post-treatment, by a validated high performance liquid chromatography method with UV detection. OFZ and fenbendazole sulphone ($FBZSO_2$) were the main metabolites detected in all three experimental groups. In animals given the 4% formulation, OFZ depleted according to a biexponential concentration vs. time curve. In contrast, those given 8 or 16% preparations produced atypical curves fitted by monoexponential equations. No statistically significant differences in area under concentration-time curves (*AUC*) were observed, but concentration-dependent differences in distribution and mean residence time (*MRT*) were evident. Compared with 4% OFZ, animals treated with 8 and 16% formulations had slower half-lives of metabolite formation, and lower *AUC's*, suggesting that OFZ sulphonation may have been modified. *In vitro* there was evidence of plasma saturation associated with 8 and 16% OFZ preparations. It is concluded that differences in PK profiles were related to OFZ solubility and/or tissue drug precipitation.

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INTRODUCTION

Benzimidazole (BZD) compounds are used to treat parasitic diseases in humans and domestic animals worldwide. For a variety of drugs physical and chemical interactions, dilution effects and/or pH modifications in pharmaceutical formulations or in different body compartments can affect their absorption and distribution. BZD compounds are all relatively insoluble in water, benzene and ether, but highly soluble in alcohol and nonpolar solvents (Towsend & Wise, 1990) and this limits the practical use of the most potent BZD compounds, including fenbendazole (FBZ), oxfendazole (OFZ) and albendazole (ABZ), to suspensions which are most commonly administered by the oral route in domestic animals. *In vivo* studies have demonstrated that the oral absorption of BZD compounds depends on their dissolution at low pH, and is closely related to the rate of gastric emptying and gut transit time (Lanusse & Prichard, 1993; Sánchez *et al.*, 1999). Fasting, dietary composition and formulation differences have all been shown to affect the pharmacokinetic (PK) behaviour and resultant efficacy of BZD compounds in mammals. A rich fatty diet improves ABZ absorption in humans (Edwards & Breckenridge, 1988), although the same effect was not observed in dogs fed with high fat diets (McKellar *et al.*, 1993). However, ABZ tablet formulations have consistently been shown to be better absorbed than lipidic matrix formulations in both dogs (Sánchez *et al.*, 2000a) and humans (Savio *et al.*, 1998). Alterations in the PK profiles of BZD compounds related to the type of diet and/or fasting have also been demonstrated in ruminants (Hennessy *et al.*, 1995; Sánchez *et al.*, 1999, Sánchez *et al.*, 2000b) and horses (McKellar *et al.*, 2002). In an attempt to improve absorption, new BZD formulations have recently been evaluated in humans using cyclodextrins as novel excipients (Díaz *et al.*, 1998; García *et al.*, 2003).

Jung *et al.* (1998) characterized ABZ and its active metabolite albendazole sulphoxide (ABZSO) as amphoteric molecules

having two ionization forms with pKa values of 10.20 and 2.80 for ABZ, and 9.79 and 0.20 for ABZSO. The chemistry of FBZ and its active metabolite OFZ (FBZ sulphoxide) is very similar to that of ABZ and its metabolites.

In an attempt to maximize systemic availability and, in turn, to improve the practical veterinary use of BZD compounds, novel injectable formulations of ABZSO and OFZ prepared in low pH solutions have recently been licensed in several South American countries for use in beef and dairy cattle. However, relationships between pH-dissolution, PK and efficacy have not yet been fully investigated. Preliminary evidence from equine studies indicated that intravenous (i.v.) administration of a concentrated (50%) suspension of OFZ produced an unexpected and atypical PK profile reminiscent of that seen following extravascular administration (McKellar *et al.*, 2002).

The aim of the present study was to evaluate the disposition kinetics of OFZ and its chiral enantiomers following i.v. administration at different (4, 8 and 16% total OFZ) concentrations. A complementary *in vitro* plasma OFZ dissolution study was also carried out.

MATERIAL AND METHODS

In vivo PK study

In a parallel experimental design, 18 parasite-free, female Suffolk sheep (40–50 kg liveweight) were allocated into three groups ($n = 6$) and treated with OFZ, formulated in dimethyl sulphoxide (DMSO), as follows: Group A, received OFZ prepared as a 4% solution at a dose of 5 mg/kg liveweight, intravenously. Groups B and C were treated with 8 and 16% OFZ preparations at the dose rate, i.e. 5 mg/kg liveweight. Heparinized whole blood was taken, by jugular venepuncture, for PK analysis, at time 0 (blank sample), 10,15, 30, 45 min and 1–4, 6, 8, 10, 12, 18, 24, 30, 36, 48, 72 h post-treatment. Plasma was obtained by centrifugation (2000 $\boldsymbol{g} \times 10$ min) and stored frozen at approximately −20 °C until analysis.

In vitro solubility test

To simulate the *in vivo* study, an absolute calculated amount of OFZ (mg) was added to plasma *in vitro* to proportionately replicate, based on a mean liveweight of 40 kg, the *in vivo* dose of 5 mg/kg liveweight used in the PK study. Assuming a 7% blood volume, *in vivo*, this equated to adding 200 mg OFZ to 2.8 L plasma. For the *in vitro* study calculated proportionate amounts of 4, 8, 16, 32 and 50% solutions of OFZ in DMSO (i.e. 35.7, 17.8, 8.9, 4.5 and 2.25 μL, respectively) were added to 20 mL of plasma. The plasma/OFZ mixtures ($n = 6$ for each formulation) were stirred for 30 min at 38 °C (i.e. body temperature), then 1 mL portions were taken from each preparation and, after centrifugation (3000 $\boldsymbol{g} \times 10$ min), the supernatants were extracted and analysed by high performance liquid chromatography (HPLC) as described below.

Plasma drug analysis

Oxfendazole, FBZ and FBZ-sulphone ($FBZSO_2$) were isolated from plasma by solid phase extraction (SPE) using C_{18} (Bond Elut®, Varian UK Ltd, Crawley, UK) cartridges with analysis of racemic achiral OFZ in plasma extracts performed using a 150 × 4.6 mm, C_{18} column (Nemesis®, Phenomenex Ltd, Macclesfield, UK) with UV detection at 296 nm, as previously described by Sánchez *et al.* (2002). Authentic standards (FBZ, batch No. D383; OFZ, batch No. A 011; $FBZSO_2$ batch No. MR 12972) for the method validation were obtained from Hoechst, AG, Frankfurt, Germany. Oxibendazole (OXB), which was used as an internal standard, was purchased from Sigma UK Ltd (Cat No. O3132). Retention times for the authentic OXB, OFZ, FBZ and $FBZSO_2$ standards were approximately 4.2, 2.1, 6.1 and 3.0 min, respectively. Calibration curves for normal ovine plasma, fortified with authentic standards, were constructed over a concentration range of 0.025–1.0 μg/mL for OFZ, FBZ and $FBZSO_2$, with a fixed concentration (1.0 μg/mL) of OXB as internal standard. Regression analysis of concentration vs. mean ($n = 3$) test analyte:internal standard peak area ratios showed good linearity with Pearson correlation coefficients >0.999 for all three test analytes. Accuracy and precision were checked at 0.025, 0.5 and 1.0 μg/mL. Mean accuracy (expressed as the mean ± SD, $n = 3$ at each concentration, percentage relative recovery calculated as: Test analyte:internal standard peak area ratio in fortified plasma extract/Test analyte:internal standard peak area ratio of equivalent authentic standards × 100) was 94.0 ± 1.6, 93.6 ± 2.2 and 100.2 ± 2.3%, respectively for OFZ, FBZ and $FBZSO_2$. Repeatability (intra-assay variation) in plasma extracts ($n = 3$) spiked at each of the same three concentrations (expressed as the mean percentage residual standard deviation, %RSD ± SD) was 5.4 ± 4.2, 3.7 ± 1.9 and 6.0 ± 3.3%, respectively for OFZ, FBZ and $FBZSO_2$. Mean intermediate precision (reproducibility, inter-assay variation), estimated as the %RSD ± SD in plasma samples spiked at 0.025, 0.5 and 1.0 μg/mL extracted and analysed individually on four separate days, was 7.7 ± 1.9, 3.4 ± 1.7 and 3.2 ± 1.2%, respectively for OFZ, FBZ and $FBZSO_2$. Mean ± SD ($n = 3$) absolute peak areas, for OFZ, FBZ and $FBZSO_2$ at 0.025 μg/mL were, respectively, 4090 ± 270, 3523 ± 430 and 2393 ± 101 compared with mean ± SD ($n = 3$) blank background noise values of 471 ± 116, 379 ± 176 and 220 ± 50, which equated to mean signal:noise ratios of 8.7, 9.3 and 10.9, respectively Whilst no claim of compliance is made the results described fulfil acceptance criteria (Precision, <20%; Accuracy, 80–120%; Signal: noise ratio, >5) prescribed by the US Food and Drug Administration (FDA) (see Guidance for Industry – Bioanalytical Method Validation, May 2001) and, on this basis a limit of quantitation (LOQ) was nominally set at 0.025 μg/mL for all three test analytes. Limits of detection (LOD) were calculated, as $3.3\ \sigma/S$ where σ = residual standard deviation of the regression lines and S = slope of the calibration curves, according to the International Cooperation on Harmonization of Technical Requirements for Registration of Veterinary Medicinal Products (VICH), Topic GL2 (Validation Methodology): Validation

of Analytical Procedures: Methodology (1998) guidelines. This resulted in estimated LOD's of 0.014, 0.013 and 0.012 μg/mL for OFZ, FBZ and $FBZSO_2$, respectively. However, for PK analysis, values below the assigned LOQ were not included.

Chiral analysis of OFZ

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). Percentages of the peak areas of OFZ enantiomers [(−)OFZ1 and (+)OFZ2] were estimated using a 100 × 4.0 mm, 5 μ,α-glycoprotein analytical column (Chiral-AGP, Chrom-Tech Ltd, Congleton, UK) in an isocratic HPLC system (Waters® 625 pump/controller; Waters® 717plus autosampler, Waters Ltd., Elstree, UK) using water/acetonitrile, 93:7, as mobile phase. The (−) and (+)OFZ were detected by UV (Waters® 486 UV detector) at 296 nm. Retention times were approximately 6.1 and 7.8 min, respectively for the (−) and (+)OFZ enantiomers.

PK analysis

PK analysis of plasma concentration vs. time data for OFZ and $FBZSO_2$ was performed using a custom computer software package (PK Solutions® 2.02, Summit Research Services, Ashland, OH, USA). The concentration vs. time curves for OFZ and $FBZSO_2$ in plasma were best-fitted by a biexponential curve (OFZ $r^2 = \sim 0.99$) for the 4% formulation and monoexponential curves for the 8 and 16% preparations ($r^2 = 0.98$–0.99) supported by the following equations (see Gibaldi & Perrier, 1982):

$$\mathrm{Cp(OFZ\ 4\%)} = C_1 e^{\lambda_1 t} + C_2 e^{\lambda_1 t},$$

$$\mathrm{Cp(OFZ\ 8\%, 16\%)} = C_2 e^{\lambda_2 t}.$$

where Cp is the plasma concentration at time, C_1 and C_2 are distribution and elimination coefficients and λ_1 and λ_2 the slopes of the distribution and elimination phases.

Elimination half-lives for OFZ and $FBZSO_2$ were calculated as $\ln 2/\beta$. The total body clearance of OFZ in plasma (Cl_b) was calculated using the conventional equation described by Gibaldi and Perrier (1982): $Cl_b = \mathrm{Dose}/AUC$. The apparent volume of distribution (Vd_{area}) was estimated as:

$$Vd_{area} = \mathrm{Dose}/\mathrm{AUC} * \lambda_2.$$

The maximum concentrations (C_{max}) and time to C_{max} (T_{max}) for $FBZSO_2$ obtained in plasma following i.v. administration of OFZ were extrapolated from the plotted concentration vs. time curve for each animal. Area under concentration-time curves (*AUCs*) were calculated according to the trapezoidal method (see Gibaldi & Perrier, 1982), with mean residence times (*MRT*) calculated as $MRT = AUMC/AUC$ where *AUC* is the area under the concentration vs. time curve from 0 to ∞ (calculated by trapezoidal method) and *AUMC* is the area under the curve of the product of time and the plasma drug concentration vs. time from 0 to ∞.

Statistical analysis

Mean plasma PK variables obtained for all groups were statistically compared by nonparametric analysis, using the Kruskal–Wallis test. Means were considered significantly different at $P < 0.05$. All PK variables are reported as the geometric mean ± SD except elimination half-lives and *MRT*, which are reported as the harmonic mean. Student's *t*-test was used for statistical comparison of enantiomeric antipode ((+)/(−)OFZ concentrations. Dunn's multiple comparison was used for statistical evaluation of the *in vitro* assay results.

RESULTS

Figures 1a–c and 2 summarize the plasma concentration depletion curves for OFZ (for up to 36 h) and $FBZSO_2$ (for up to 72 h), respectively, in sheep, after i.v. administration of 4, 8, and 16% preparations of OFZ. Traces of FBZ were detected after OFZ administration in all three experimental groups but values were <LOQ and, therefore, the data is not included. As illustrated, the pattern of depletion of OFZ differed depending on the concentration of the formulation given. This was reflected both by alterations in the shape of the depletion curves, which were related to marked and concentration-dependent differences in *MRT* (Table 1). *MRT* was significantly ($P < 0.05$) longer for the 16% compared with the 4% formulation. Other plasma PK parameters for OFZ and its inactive metabolite, $FBZSO_2$, after the i.v. OFZ administration at different concentrations are summarized in Tables 1 and 2. For OFZ (Table 1), mean Cp0 was significantly lower, and Vd_{area} significantly higher, in the group given 16% OFZ compared with that given the 4% formulation. In both instances values for the group given 8% OFZ were intermediate. There were no statistically significant, dose-related differences in λ_2, $t_{1/2}\lambda_2$, *AUC* and *AUMC* or Cl_b. For $FBZSO_2$ (Table 2), group mean values for $t_{1/2}\lambda_1$ ($P < 0.05$), $t_{1/2}\lambda_2$ ($P < 0.001$) and *MRT* ($P < 0.01$) were all significantly higher, and C_{max} ($P < 0.05$) and *AUC* ($P < 0.05$) significantly lower, following administration of the 16% compared with the 4% preparation. Although, for these parameters the 8% OFZ formulation was associated with intermediate values, for *AUMC* the lowest mean value was recorded in this group. The result was not significantly different from that given by the 4% OFZ group, but it was significantly ($P < 0.05$) lower than that calculated for the 16% OFZ group.

Concentration depletion curves for the (−) and (+)OFZ enantiomers in the three treatment groups, plotted from data obtained by chiral HPLC analysis, are shown as insets to Fig. 1a–c. The *AUCs* for the (−) and (+)OFZ enantiomers, calculated from these curves, showed a parallel depletion from plasma, representing 50% of the total *AUC* values calculated for the racemic analysis. The plasma distribution ratio of (+):(−)OFZ enantiomers for the 4% OFZ treatment group increased from 1.02 to 4.44 over 36 h

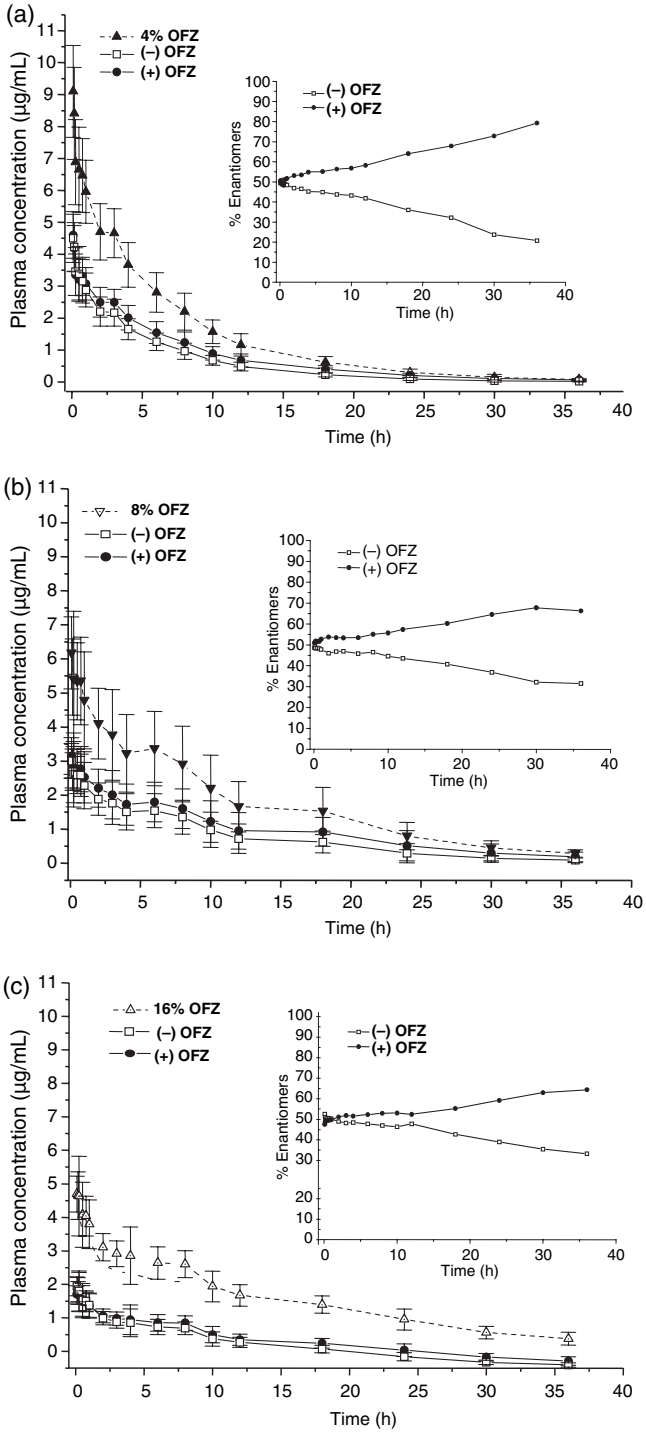

Fig. 1. The larger plots show the plasma depletion profiles for total oxfendazole (OFZ) (plotted as mean plasma concentration ± SD, $n = 6$) in sheep monitored for 36 h after the i.v. administration of 4% (Fig. 1a), 8% (Fig. 1b) or 16% (Fig. 1c) preparations of OFZ at a dose rate of 5 mg/kg liveweight. The insets in Fig. 1a–c show the relative distribution of the (−) and (+)OFZ enantiomers during the same 36 h post-treatment period.

post-treatment. In contrast, ratios of the (+):(−)OFZ enantiomers for the 8 and 16% OFZ preparations increased from 1.04 to 2.11 and from 0.90 to 1.94, respectively, over the same period.

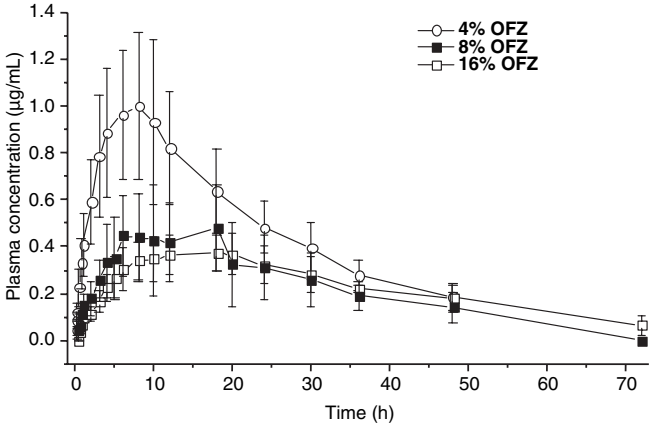

Fig. 2. Plasma concentration curves of fenbendazole sulphone ($FBZSO_2$) (plotted as mean plasma concentration ± SD, $n = 6$) in sheep monitored for 72 h after i.v. administration of 4, 8 and 16% preparations of oxfendazole (OFZ) at a dose of 5 mg/kg.

Concerning the results obtained in the *in vitro* dissolution test, highest dissolution was obtained at the lowest (4%) OFZ concentration tested. This observation correlated with the elevated *in vivo* plasma OFZ concentration detected at time 0 (Cp0) in the 4% OFZ treatment group compared with those obtained for the 8 and 16% OFZ preparations (Table 1 and Fig. 3).

DISCUSSION

Aqueous solubility plays an important role in drug formulation and for oral administration it is widely accepted that, unless the substance has an aqueous solubility above 10 mg/mL over a pH range of 1–7, then potential absorption problems may occur. Solubility of less than 1 mg/mL is likely to give dissolution-rate absorption equivalence because solubility and dissolution rate are directly related (Kristensen, 1996). As a consequence, in order to achieve high and sustained concentrations at gastric sites of parasite infection, high dissolution rates are required at low pH for oral BZD formulations. Enhancement of the absorption and distribution processes is correlated with the efficacy of the BZD compounds (Shastri *et al.*, 1980). The present study describes effects of dissolution rate, under experimental conditions, on the plasma PK profile of OFZ administered intravenously to sheep as 4, 8 or 16% solutions.

In agreement with a recent report (Sánchez *et al.*, 2002) it was confirmed that for OFZ administered intravenously as 4% preparation the plasma concentration-time curves for total OFZ, and its two chiral enantiomers, displayed two defined phases fitted to a bi-exponential curve (Fig. 1a). In the present study, plasma concentration at time 0 (Cp0) for 4% OFZ was found to be significantly higher ($P < 0.05$) than that obtained for the 8 and 16% formulations (Table 1). This may have been due to enhanced dissolution of this preparation; a contention supported by the observed differences in the concentration vs. time curve depletion patterns obtained *in vivo*. These findings were

Table 1. Comparative plasma pharmacokinetic (PK) parameters obtained for oxfendazole (OFZ) in groups of sheep ($n = 6$) dosed intravenously at 5 mg/kg liveweight with formulations containing 4, 8 or 16% OFZ

	OFZ 4%		OFZ 8%		OFZ 16%	
PK parameters	Mean	SD	Mean	SD	Mean	SD
Cp0 (μg/mL)	9.10*	1.43	6.18*	0.94	4.70*	0.53
λ_2 (per h)	0.13	0.01	0.11	0.01	0.08	0.02
$t_{1/2}\lambda_2$ (h)	5.52	0.39	6.45	0.72	8.72	1.98
AUC (μg·h/mL)	49.7	10.8	49.4	18.5	47.0	8.0
AUMC (μg·h^2/mL)	385	99	491	217	662	260
MRT (h)	7.93*	1.27	9.34	1.0	13.0*	3.1
Vd_{area} (L/kg)	658**	189	987	278	1245**	211
Cl_b (mL kg/h)	104	27.1	111	46.0	102	16.8

Cp0, plasma concentration at time 0; λ_2, rate constant for the elimination Phase; $t_{1/2}\lambda_2$, elimination half-life; *AUC*, area under the concentration-time curve; *AUMC*, area under the first moment curve; *MRT*, mean residence time; Vd_{area}, apparent volume of distribution; Cl_b, total body clearance.
Figures with common superscripts are significantly different (*$P < 0.05$) and (**$P < 0.01$) by Kruskal–Wallis test.

Table 2. Comparative plasma pharmacokinetic (PK) parameters obtained for fenbendazole sulphone ($FBZSO_2$) in groups of sheep ($n = 6$) dosed intravenously at 5 mg/kg liveweight with formulations containing 4, 8 or 16% OFZ

	OFZ 4%		OFZ 8%		OFZ 16%	
PK parameter	Mean	SD	Mean	SD	Mean	SD
$t_{1/2}\lambda_1$ (h)	1.71*	0.42	2.80	1.10	3.66*	1.44
C_{max} (μg/mL)	1.02*	0.33	0.53	0.19	0.40*	0.09
T_{max} (h)	7.33	1.63	8.00	3.67	12.00	3.35
AUC (μg·h/mL)	25.9*	6.1	15.4	6.7	16.2*	3.8
AUMC (μg·h^2/mL)	756*	194	520	236	1022*	244
$t_{1/2}\lambda_2$ (h)	16.4***	1.6	20.0	2.3	31.0***	8.4
MRT (h)	26.2**	2.4	29.7	4.0	48.3**	11.4

$t_{1/2}\lambda_1$, metabolite formation rate; C_{max}, peak plasma concentration; T_{max}, time at C_{max}; *AUC*, area under the concentration vs. time curve extrapolated to infinity; *AUMC*, area under the first moment concentration vs. time curve extrapolated to infinity; $t_{1/2}\lambda_2$, elimination half-life; *MRT*, mean residence time.
Figures with common superscripts are significantly different (*$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$) by Kruskal–Wallis test.

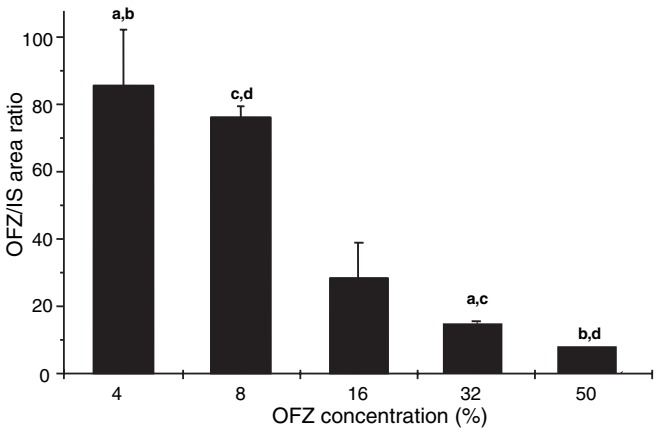

Fig. 3. *In vitro* plasma dissolution test for 4, 8, 16, 32 and 50% preparations of oxfendazole (OFZ) showing the relative mean ($n = 6$) OFZ/Internal Standard (IS) peak area ratios (±SD) after incubation for 30 min at 38 °C. Common letters indicate significant differences ((a) and (c), $P < 0.05$; (b) and (d), $P < 0.001$) between groups according to Dunn's multiple comparison test.

supported by results generated in parallel *in vitro* assays, in which markedly higher drug dissolution was found after incubation of plasma spiked with 4% (w/v) OFZ, compared with values obtained for plasma fortified with higher concentrations of OFZ. *In vivo*, atypical concentration-time curves, fitted by mono-exponential equations, were obtained for the 8 and 16% OFZ racemic preparations (Fig. 1b, c). Differences in drug depletion between the different OFZ preparations were reflected in alterations of PK parameters relating to distribution and *MRT* (Table 1). The *MRT* of OFZ following dosing with the 4% solution was significantly ($P < 0.05$) shorter (7.93 ± 0.90 h) than that obtained for the 16% OFZ preparation (13.0 ± 3.10 h). The *MRT* for the 8% OFZ preparation (9.34 ± 1.00 h) was intermediate between, but not significantly different from, these two values. Compared to the 4% treatment group, apparent OFZ distribution, as described by Vd (see Table 1), was also higher for the 8 and 16% preparations and this difference was statistically significant ($P < 0.01$) for the latter.

The low OFZ concentrations obtained *in vivo* for the 8 and 16% preparations, were similar to those associated with the

curve obtained in horses after the experimental i.v. administration of a concentrated (50%) formulation of OFZ (McKellar *et al.*, 2002). In the latter study, the concentration-time curve was comparable with that obtained after extra-vascular administration. Whilst there is no current experimental evidence, it is possible that for concentrated formulations undissolved OFZ particles present, due to saturation, are deposited as a reservoir in tissues (e.g. lung). Gradual release from such tissue deposits over time could account for the observed increases in plasma OFZ concentrations. Such a mechanism might also explain the observed differences in *MRT* and Vd, obtained for the different OFZ formulations used in the present study (Table 1). The extent of this effect is likely to be related to the degree of saturation of the different OFZ formulations examined: 4% OFZ was a solution, 8% OFZ a nonsaturated suspension and the 16–50% preparations incrementally increasing saturated suspensions.

It is well established that BZD compounds have a complex biotransformation, which has been described *in vivo* and *in vitro* in several domestic species (Souhaili-El-Amri *et al.*, 1988; Delatour *et al.*, 1991; Murray *et al.*, 1992). OFZ is the active metabolite formed by flavin monooxygenase (FMO)-mediated sulphoxidation of its parent drug, FBZ. OFZ is a chiral molecule from which it is possible to isolate two enantiomers, (−) and (+)OFZ. One of these, (−)OFZ, but not the other is converted, by irreversible sulphonation, via the cytochrome-P450 3A system, into the inactive metabolite $FBZSO_2$, and it has been suggested that the generation of $FBZSO_2$ is rate-limited by the formation of its precursor OFZ (Lanusse & Prichard, 1993). In the present study it was shown that the formation rate ($t_{1/2}\lambda_1$) of $FBZSO_2$, after the injection of 4% OFZ was more rapid than with the 8 and 16% formulations (Table 2). This is consistent with a greater availability for $FBZSO_2$ and this is also reflected in the higher (approximately 60%) *AUC* for the 4% OFZ solution, compared with the 8 and 16% preparations (Fig. 1a–c, and Table 2). Interestingly, the concentration-time curves for $FBZSO_2$ for both the 8 and 16% preparations depleted in parallel and there were no statistical differences in any of the associated PK parameters (Table 2). For the 16% OFZ preparation the *MRT* obtained for $FBZSO_2$ was similar to that obtained for OFZ and was significantly longer ($P < 0.01$) than that obtained for the 4% OFZ solution. The mechanism(s) underlying the observed concentration-dependent differences in the PK profiles of OFZ and $FBZSO_2$ are unclear although it is our hypothesis that inadequate dissolution, perhaps related to differential saturations of the formulations used and tissue drug precipitation, may be responsible.

Relative distribution profiles for the two enantiomers of OFZ for the three test total OFZ preparations were also characterized in the current study. Individual plasma depletion curves for (−) and (+)OFZ paralleled that for the racemate, and individual *AUCs* calculated for the enantiomers each represented 50% of the total *AUC* value obtained from the achiral analysis. There were, however, apparent differences plasma enantiomeric distribution ratio (i.e. (+):(−)OFZ) for the three OFZ preparations and these could have impacted on the kinetics of the OFZ sulphonation. Differences, *in vivo*, in Cp0 between the different OFZ preparations may have been associated with reduced dissolution in plasma. The observed *in vitro* plasma dissolution values for 4, 8 and 16% OFZ preparations were very different (85, 75 and 25%, respectively) and probably dependent on their relative degrees of saturation. These differences could account for the differences in Cp0 obtained for the different *in vivo* treatment groups.

In conclusion, the findings of the present study suggest that the atypical PK behaviour, associated with the i.v. administration of different concentrations of OFZ, may be related to concentration-dependent differences in saturation, dissolution, metabolism and possibly tissue deposition of undissolved and/or precipitated drug. Tissue distribution studies are currently being undertaken to further resolve this issue.

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