Albendazole sulphoxide enantiomeric ratios in plasma and target tissues after intravenous administration of ricobendazole to cattle

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The comparative concentration profiles of the (+) and (–) albendazole sulphoxide (ABZSO) enantiomers obtained in plasma and in selected target tissues/fluids after intravenous (i.v.) administration of a racemic formulation of ricobendazole (RBZ) to cattle were characterised. Fourteen Holstein calves received RBZ (racemic solution, 150 mg/mL) by i.v. administration at 7.5 mg/kg. Jugular blood samples were collected over 48 h post-treatment (plasma kinetic trial) and two animals were sacrificed at either 4, 12, 20, 28 or 32 h post-treatment to obtain samples of abomasal/small intestine mucosal tissue, abomasal/small intestine fluids, bile, liver and lung tissue (tissue distribution study). The (–)ABZSO enantiomer was depleted significantly faster from plasma compared with the (+)ABZSO antipode. The plasma *AUC* for (+)ABZSO (38.3 μg · h/mL) was significantly higher ($P < 0.05$) compared with that obtained for (–)ABZSO (20.5 μg · h/mL). The (+)ABZSO enantiomer was the predominant antipode measured in bile, abomasal fluid and abomasal mucosa. For instance, at 12 h post-treatment the (+)/(–) concentration ratios were: 12.9 (plasma), 1.62 (abomasal mucosa), 13.0 (abomasal fluid), 2.92 (intestinal mucosa), 9.87 (intestinal fluid) and 21.5 (bile). No marked differences between the concentration profiles of both enantiomers were observed in the liver tissue. Albendazole (ABZ) was recovered from the liver, lung and gastrointestinal (GI) mucosal tissues of RBZ-treated calves up to 32 h post-treatment, probably produced by a GI microflora-mediated sulphoreduction of RBZ. An enantioselective kinetic behaviour may account both for the faster depletion of the (–) enantiomer and for the higher availabilities of the (+) antipode observed in plasma and in most of the tissues/fluids investigated. The simultaneous evaluation of the plasma kinetics and tissue concentration profiles of both enantiomeric forms reported here, may help to interpret the relationship between chiral behaviour and pharmacological action for sulphoxide derivatives of benzimidazole (BZD) methylcarbamate anthelmintics.

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INTRODUCTION

Benzimidazole (BZD) and pro-BZD anthelmintics are widely used in veterinary and human anthelmintic therapy. The use of these compounds became widespread because they offered major advantages over previous available drugs in terms of spectrum, activity against immature parasitic stages and host safety (Campbell, 1990). Ricobendazole (RBZ) or albendazole sulphoxide (ABZSO), is quantitatively the most important active metabolic product recovered in the bloodstream (Prichard *et al.*, 1985; Hennessy *et al.*, 1989) and tissues/fluids (Lanusse *et al.*, 1993; Alvarez *et al.*, 1999), after administration of either albendazole (ABZ) or its pro-drug (netobimin) in cattle and sheep. Ricobendazole is also available as an anthelmintic compound for use in ruminant species as an oral preparation, and more recently as an injectable formulation for use in cattle (Lanusse *et al.*, 1998).

This BZD-sulphoxide derivative is a chiral molecule with an asymmetric centre in the sulphur atom, which is attached to four different functional groups; this results in an asymmetric

molecule nonsuperimposable with its mirror image. Two ABZSO enantiomers have been identified in plasma after oral administration of ABZ (Delatour *et al.*, 1991). Although the plasma concentration profiles of ABZSO enantiomers have been characterised in many species (Delatour *et al.*, 1991; Lanchote *et al.*, 1998), no information is available on the relative distribution of these molecules to different tissues where parasites are located. The antiparasitic activity of BZD moieties depends largely on their ability to reach high and sustained concentrations at the site of parasite location, which depends on pharmacokinetic, metabolic and tissue distribution processes in the host. Thus, characterisation of the disposition kinetics and distribution of ABZSO enantiomers to selected target tissues may contribute to understanding the relationship between chiral behaviour and anthelmintic activity of BZD methylcarbamates. In the experimental work reported here, the comparative enantiomeric behaviour of ABZSO in plasma and in selected target tissues/fluids was characterised following the intravenous (i.v.) administration of a racemic (50% of each enantiomer) formulation of RBZ to cattle.

MATERIALS AND METHODS

Animals

Parasite-free, healthy male Holstein calves (130–140 kg) were used in this experimental work. Animals were fed with high quality hay and water provided *ad libitum.*

Plasma disposition study

Four calves were treated with a racemic (50% of each enantiomer) injectable solution of RBZ (Bayverm PI®, 150 mg/mL, Bayer, Argentina) by i.v. injection in the right jugular vein at 7.5 mg/kg. Blood samples were taken into heparinised tubes from the left jugular vein prior to treatment and at 2.5, 5, 10, 15, 30 min, 1, 2, 4, 6, 8, 10, 12, 16, 20, 24, 28, 32, 36 and 48 h after drug administration. Blood samples were centrifuged at 3000 ***g*** for 15 min. The collected plasma samples were stored at –20 °C until chromatographic analysis.

Tissue distribution study

Ten animals were treated with the same RBZ racemic formulation by the same route and dose rate. Two treated calves were killed at 4, 12, 20, 28 and 32 h postadministration. Calves were stunned by captive bolt and exanguinated immediately according to internationally accepted animal welfare guidelines (Canadian Council on Animal Care (CCAC), 1980; American Vertinary Medical Association (AVMA), 1993). Samples of blood, liver, lung tissue, abomasal and small intestine mucosal tissue and fluid contents and bile were obtained from each animal. One untreated animal was killed to obtain 'blank' samples to validate the analytical methodology. Blood samples were centrifuged at 3000 ***g*** for 15 min to obtain plasma. The contents of the abomasum and upper small intestine were collected, thoroughly mixed and subsamples obtained; they were centrifuged at 4000 ***g*** and the supernatants (fluid and fine particulate digesta) recovered, stored into polypropylene tubes at 5 °C and transported to the laboratory. After collection of the abomasal/small intestine contents, the mucosal tissue was washed and samples of the abomasal and small intestine (upper section) mucosal tissues were obtained by scraping; the scrapped mucosal material was thoroughly mixed and representative aliquots obtained for analysis. Bile was collected directly from the gall bladder at the time of killing. Liver and lung tissue samples were collected into labelled plastic bags. All the experimental samples collected were stored at –20 °C until the time of analysis.

Sample extraction and chromatographic analysis

Aliquots (0.5 mL) of the collected plasma, bile, abomasal and small intestine fluid samples were supplemented with oxibendazole (OBZ) (as internal standard, 99.2% pure, 0.5 μg/5 μL methanol) and 1 mL acetonitrile. They were subjected to chemical extraction and clean up processes as previously described (Alvarez *et al.*, 2000), using a Gilson ASPEC XL Sample Processor for Solid Phase Extraction (GILSON®, Middleton, USA). After elution, the samples were evaporated to dryness under a stream of N_2. The dry residue was redissolved in 300 μL mobile phase and 50 μL were injected into the high performance liquid chromatography (HPLC) system. Drug/metabolite extraction from tissue samples was carried out using the matrix solid phase dispersion technique previously reported (Alvarez *et al.*, 2000).

Experimental and fortified plasma, bile, gastrointestinal (GI) fluid/mucosa, liver and lung tissue samples were analysed for ABZ, ABZSO and ABZ sulphone ($ABZSO_2$) using HPLC. Fifty microlitres (50 μL) were injected into a Shimadzu 10 A HPLC system (Shimadzu Corporation, Kyoto, Japan) fitted with a Selectosil C_{18} (5 μm, 250 × 4.60 mm) reverse-phase column (Phenomenex, CA, USA) and UV detector (SPD-10A, Shimadzu Corporation, Kyoto, Japan) at 292 nm, the analytical conditions were as previously reported (Alvarez *et al.*, 1999, 2000). The analytes were identified with the retention times of 97–99% pure reference standards. Under these chromatographic conditions the retention times were 5.9 min (ABZSO), 7.4 min ($ABZSO_2$), 10.3 min (OBZ, used as internal standard) and 12.9 min (ABZ). The peak areas of each analyte were measured using the Shimadzu Class LC 10 (Version 1.12, 3.86 CBM-10A, Shimadzu Corporation, Kyoto, Japan) software on an IBM compatible computer. The quantification limit for all the analytes was 0.01 μg/g in the different fluids and tissues analysed; this value was the lowest concentration detected with a coefficient of variation (CV) lower than 20%. Experimental concentration values below the quantification limit were not used for the pharmacokinetic analysis of the data. Drug and metabolite recoveries were established by comparison of the detector responses obtained for fortified blank samples and those of direct standards prepared in mobile phase. Recovery percentages

ranged from 84.3 to 91.8% for the different analytes in the various tissues/fluids investigated. Calibration curves for each analyte were prepared from the least squares linear regression analysis of HPLC peak area ratios of unknown analytes/internal standard and nominal concentrations of spiked tissues/fluids samples (0.01–5 μg/mL or μg/g). When concentrations were outside the calibration line range, the analysis of the samples was repeated using half amount of tissue sample. There was no interference of endogenous compounds in the chromatographic determinations.

Enantiomeric separation

During the reverse phase HPLC analysis, the ABZSO peak fraction was collected into a glass tube at its retention time of 5.9 min. The collected fraction was evaporated to dryness under a N_2 stream and redissolved with 150 μL of chiral mobile phase (1% 2-propanol in 8 mM of Na_2HPO_4 buffer, pH 6.9). Fifty microlitres (50 μL) of each sample were injected into the same HPLC system fitted with a chiral stationary phase column (5 μm, 100 × 4.0 mm) (Chiral-AGP, ChromTech, Hägersten, Sweden). This chiral chromatographic method was adapted from that described by Delatour *et al.* (1990). Albendazole sulphoxide enantiomers were identified after chromatographic analysis of a 99.5% racemic standard of this molecule. The retention times of both enantiomers were 3.4 min for (–)ABZSO and 6.0 min for (+) ABZSO. The relative proportions (%) of both enantiomers in the different tissues/fluids were obtained using the integrator software (Class LC10, version 1.62, 3.86 CBM-10A, Shimadzu Corporation, Kyoto, Japan) of the HPLC system.

Pharmacokinetic and statistical analysis

The concentration vs. time curves estimated for each ABZSO enantiomer and the sulphone metabolite in plasma were fitted with the PkSolutions 2.0 computer program (Ashland, OH, USA) (Farrier, 1997). The plasma disposition kinetics of both ABZSO enantiomers and $ABZSO_2$ was described following routine equations for noncompartmental methods of analysis (Gibaldi & Perrier, 1982). The ratio between the concentrations of the (+) and (–) enantiomeric forms of ABZSO at different times post-treatment was estimated in the different tissues and fluids investigated. Pharmacokinetic parameters are presented as mean ± SD. The values for the pharmacokinetic parameters collected for both ABZSO enantiomers in plasma were statistically compared using the Wilcoxon nonparametric test. A value of $P < 0.05$ was considered statistically significant.

RESULTS

The plasma kinetics of each ABZSO enantiomer collected after the i.v. injection of a racemic formulation of RBZ to cattle was markedly different. The (+)ABZSO enantiomer was detected in plasma between 2.5 min and 32 h post-treatment, while the (–) antipode was measured in the bloodstream between 2.5 min and 16 h after treatment. The comparative mean plasma concentrations vs. time plots for both enantiomers are shown in Fig. 1. Both ABZSO enantiomers displayed a similar disposition pattern with a rapid distribution phase and a slow elimination phase. However, the (–) enantiomer was depleted significantly ($P < 0.05$) faster ($T½_{el} = 3.02$ h) from plasma compared with the (+) antipode ($T½_{el} = 5.39$ h). The plasma *AUC* for the (+) enantiomer was 81% more than that obtained for (–)ABZSO. The parameters describing the comparative plasma disposition of both enantiomeric forms after i.v. administration of racemic RBZ are summarised in Table 1. The $ABZSO_2$ metabolite was measured in plasma between 2.5 min and 28 h

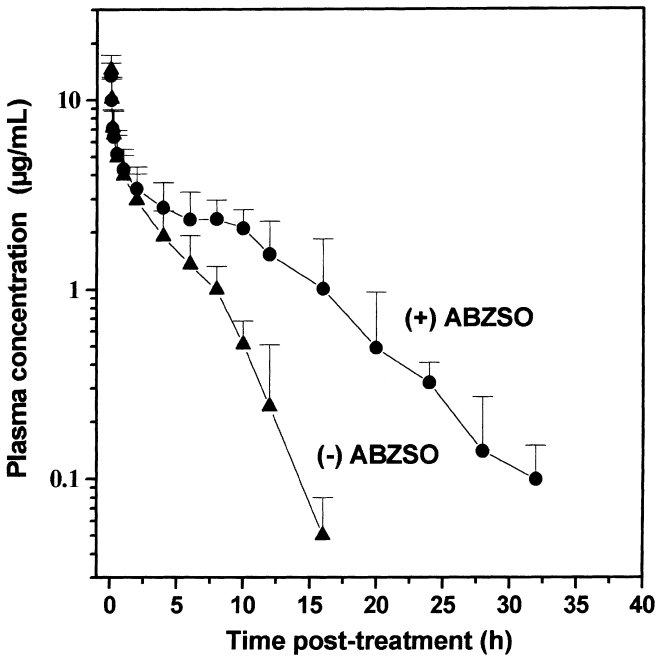

Fig. 1. Mean (±SD) plasma concentrations of (+) and (–) albendazole sulphoxide (ABZSO) enantiomers obtained after the intravenous administration (7.5 mg/kg) of racemic ricobendazole (RBZ) to cattle.

Table 1. Mean (±SD) plasma pharmacokinetic parameters for (+) and (–) albendazole sulphoxide (ABZSO) enantiomers obtained after intravenous administration (7.5 mg/kg) of a racemic formulation of ricobendazole (RBZ) to cattle

	(+)ABZSO	(–)ABZSO
k_{el} (h^{-1})	0.13 ± 0.01	0.23 ± 0.05*
$T½_{el}$ (h)	5.39 ± 0.53	3.02 ± 0.98*
$AUC_{0-\infty}$ (μg h/mL)	38.3 ± 9.62	20.5 ± 3.60*
MRT (h)	7.60 ± 0.47	4.28 ± 1.37*
Vd_{ss} (l/kg)	0.79 ± 0.17	0.85 ± 0.46
Cl_B (ml/h kg)	103 ± 32.0	199 ± 51.1*

*Values are significantly different from those obtained for the (+) enantiomer at $P < 0.05$.

k_{el}, elimination rate constant; $T½_{el}$, elimination half-life; $AUC_{(0-\infty)}$, area under concentration–time curve extrapolated to infinity; MRT, mean residence time; Vd_{ss}, volume of distribution at the steady state; Cl_B, total body clearance.

Table 2. Mean (±SD) kinetic parameters describing the disposition kinetics of albendazole sulphone ($ABZSO_2$) after intravenous administration (7.5 mg/kg) of a racemic formulation of ricobendazole (RBZ) to cattle

	$ABZSO_2$
C_{max} (μg/mL)	2.20 ± 0.30
T_{max} (h)	13.0 ± 3.50
$AUC_{0-\infty}$ (μg h/mL)	29.5 ± 3.46
$T½_{el}$ (h)	2.28 ± 0.43
MRT (h)	11.5 ± 0.74

C_{max}, peak plasma concentration; T_{max}, time to peak concentration; $AUC_{(0-\infty)}$, area under concentration–time curve extrapolated to infinity; $T½_{el}$, elimination half-life; MRT, mean residence time.

postadministration of RBZ, reaching a peak concentration of 2.20 μg/mL at 13.0 h post-treatment. The plasma kinetic parameters obtained for $ABZSO_2$ are presented in Table 2.

Both ABZSO enantiomers and $ABZSO_2$ were measured in the different fluid and tissue samples analysed. The comparison of the concentration profiles of (+) and (–)ABZSO enantiomers in abomasal fluid and mucosa is shown in Fig. 2. As described for plasma, (+)ABZSO was the enantiomeric form recovered at the highest concentration, both in the fluid content and mucosal tissue of the abomasum at all post-treatment sampling times. Concentrations of (+)ABZSO in abomasal mucosa were between 62 and 352% more than those measured for the (–) antipode. The comparative concentrations of both enantiomers in the fluid and mucosa of the upper small intestine are shown in Fig. 3.

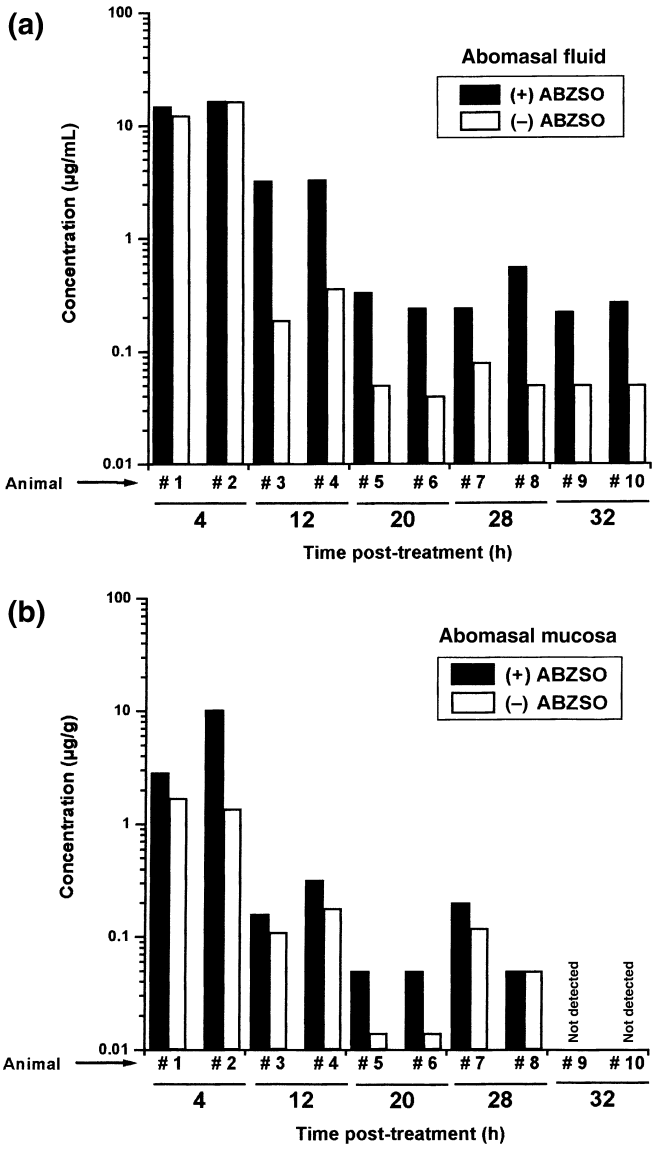

Fig. 2. Comparative concentration profiles of albendazole sulphoxide (ABZSO) enantiomers in abomasal fluid (a) and mucosal tissue (b) following the intravenous (7.5 mg/kg) administration of racemic ricobendazole (RBZ) to cattle.

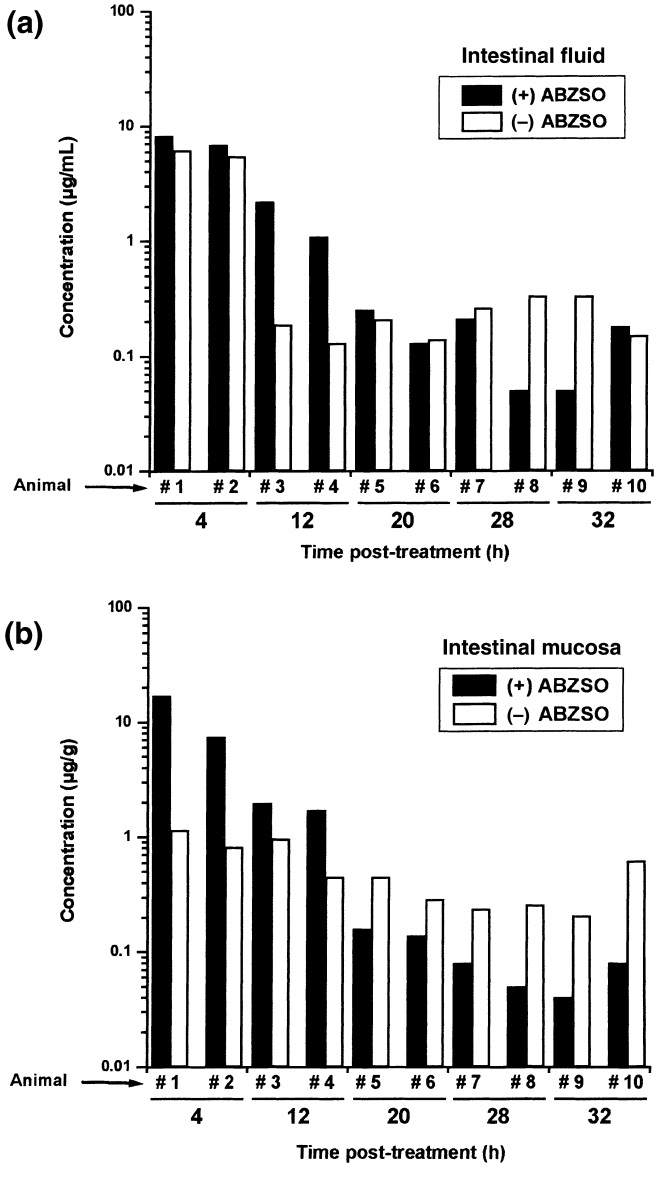

Fig. 3. Comparative concentration profiles of albendazole sulphoxide (ABZSO) enantiomers in small intestinal fluid (a) and mucosal tissue (b) following the intravenous (7.5 mg/kg) administration of racemic ricobendazole (RBZ) to cattle.

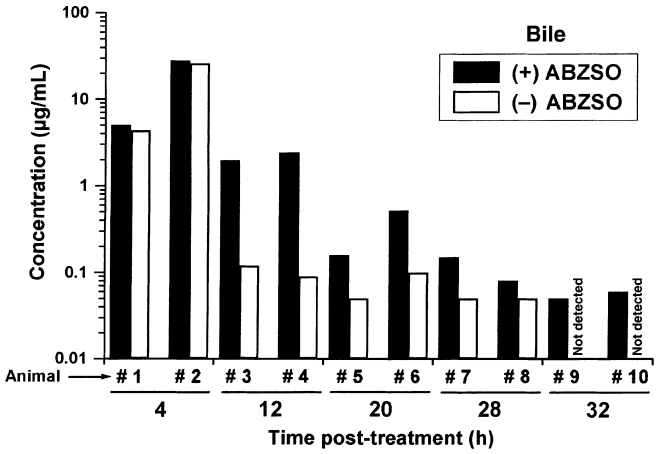

Fig. 4. Comparative concentration profiles of albendazole sulphoxide (ABZSO) enantiomers measured in bile following the intravenous (7.5 mg/kg) administration of racemic ricobendazole (RBZ) to cattle.

Although the concentrations of the (+) enantiomer in the small intestine (mucosa and fluid) appeared to be higher for up 12 h post-treatment, (–)ABZSO prevailed in the latest sampling points.

The comparative concentration profiles of (+) and (–)ABZSO measured in bile are shown in Fig. 4. The (+) enantiomeric form was the main analyte recovered in bile between 12 and 32 h post-treatment; bile concentrations of (+)ABZSO ranged between 28.3 (at 4 h post-treatment) and 0.05 µg/mL (32 h). While the (–) antipode was not detected at 32 h post-treatment, bile concentrations of (+)ABZSO were 21.5- (12 h), 4.33- (20 h) and 2.30-fold (28 h) higher than those measured for (–)ABZSO at the same sampling times. The comparisons of the (+)ABZSO/(–)ABZSO concentration ratios obtained in plasma, GI mucosa/fluids and bile are shown in Table 3. Overall, these results demonstrate that (+)ABZSO is the predominant enantiomeric form recovered in plasma, abomasum (fluid and mucosa) and bile throughout the experimental period. The same is valid for the small intestine mucosa during the first 12 h postinjection of racemic RBZ. Although equivalent proportions of both antipodes were measured at the earliest sampling times, the concentrations of (–)ABZSO measured in the liver between 20 and 32 h post-treatment tended to be slightly higher. Both enantiomeric forms were recovered in the lung tissue up to 20 h post-treatment, reaching peak concentrations of 2.06 [(+)ABZSO] and 0.27 µg/g (–)ABZSO] at 4 h post-treatment.

Albendazole obtained as a sulphoreductive metabolic product, was recovered from the liver, abomasal and small intestine mucosa/fluids of RBZ-treated calves up to 32 h postadministration. The concentration profiles of ABZ measured in those target tissues are shown in Fig. 5. This molecule was also recovered in bile and small intestine content fluid (data not shown) and the lung tissue (up to 20 h post-treatment). Large quantities of ABZ (12.1 µg/g at 4 h post-treatment) were measured in abomasal mucosa after RBZ administration. While ABZSO was the predominant analyte recovered in the GI fluids, ABZ was the main molecule measured in the digestive tract mucosal tissues. The relative quantities of ABZ and total ABZSO recovered in the mucosa and fluid of the abomasum are shown in Table 4.

DISCUSSION

The length of time in which parasites are exposed to toxic drug concentrations is a major descriptor of the clinical efficacy of BZD anthelmintics (Lacey, 1988). It has been shown that plasma concentration profiles of ABZ metabolites reflect the pattern of exposure of helminths in the digestive tract (Alvarez *et al.*, 1999). The binding of ABZ to plasma proteins is <50%, which agrees with the values of volume of distribution previously described. In fact, large volumes of distribution for ABZ and its metabolites have been reported in sheep (Galtier *et al.*, 1991) and cattle (Sánchez *et al.*, 1997). The volume of distribution for the enantiomeric forms reported here ranged between 0.79 [(+)ABZSO] and 0.85 L/kg [(–)ABZSO], which indicates that both antipodes may have a similar distribution pattern to reach parasite location tissues. However, these values are lower than those reported for ABZ (3.4 L/kg) after its i.v. administration to cattle (Sánchez *et al.*, 1997). Although these differences between both ABZSO enantiomeric forms and ABZ may be consequence of many factors, including differential affinity for plasma proteins, the markedly higher lipid solubility of ABZ compared with ABZSO may facilitate its more extensive distribution to peripheral tissues when both compounds are intravenously administered. This differential pattern of distribution is in agreement with the larger amounts of ABZ, compared with ABZSO, recovered in tapeworms collected from i.v. ABZ-treated sheep (Alvarez *et al.*, 1999).

After oral/intraruminal (i.r.) administration of ABZ to cattle, the parent compound is undetectable in plasma, while ABZSO

Table 3. Ratios between the concentrations of the (+) and (–) albendazole sulphoxide (ABZSO) enantiomers measured in different tissues/fluids following intravenous administration (7.5 mg/kg) of racemic ricobendazole to cattle

Time post-treatment	Plasma	Abomasal mucosa	Abomasal fluid	Intestinal mucosa	Intestinal fluid	Bile
4 h	1.41	4.52	1.10	11.8	1.29	1.13
12 h	12.9	1.62	13.0	2.92	9.87	21.5
20 h	3.12	3.57	6.30	0.42	1.06	4.33
28 h	ND	1.66	7.00	0.26	0.48	2.30
32 h	ND	ND	4.90	0.16	0.68	ND

ND: not determined because of the lack of detection of the (–) ABZSO enantiomer.
Data represent the ratio between mean ($n = 2$) concentration values of the (+) and (–) ABZSO enantiomers measured in each tissue/fluid at different times post-treatment.

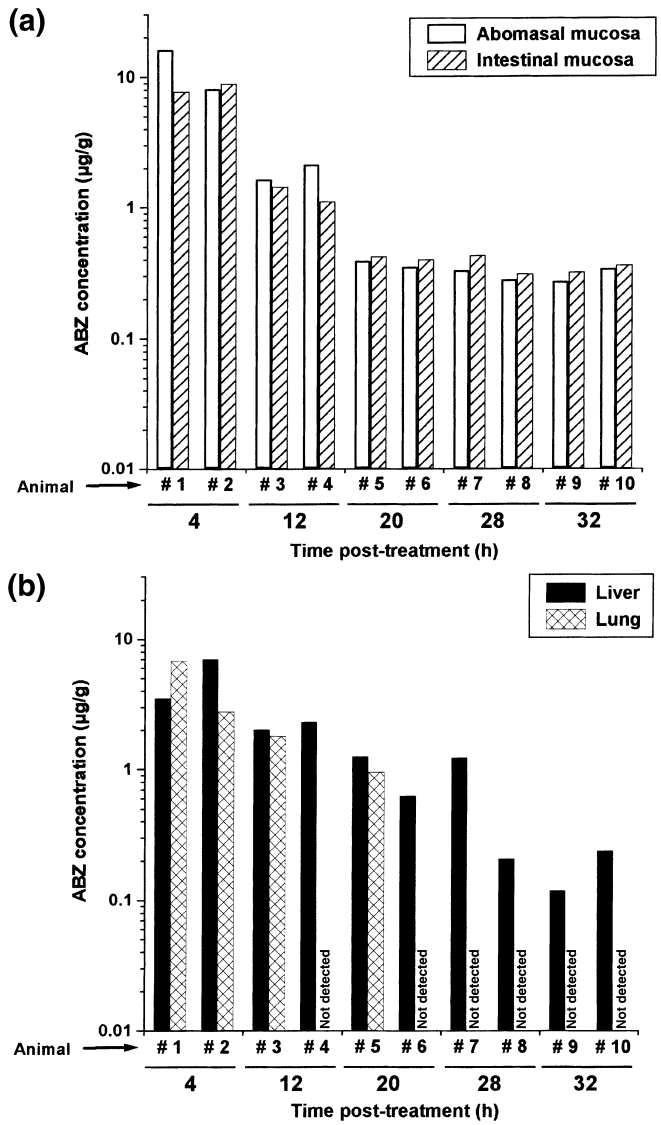

Fig. 5. Concentrations of albendazole (ABZ) measured in the mucosa of abomasum and upper small intestine (a), liver and lung tissue (b) following the intravenous (7.5 mg/kg) administration of ricobendazole (RBZ) to cattle.

and $ABZSO_2$ are the major analytes recovered in the bloodstream (Prichard *et al.*, 1985; Sánchez *et al.*, 1997). The absence of ABZ in plasma has been attributed to a first-pass oxidation in the liver. The (+)ABZSO enantiomer represents 91% of the total ABZSO plasma area under the concentration vs. time curve in cattle (Delatour *et al.*, 1991). This enantioselective kinetic profile was attributed to the relative contribution of the flavin containing monooxigenase and cytochrome P450-dependent oxygenases to ABZ sulphoxidation and sulphonation. In agreement with those results, the plasma disposition kinetics of both ABZSO enantiomers, obtained after the i.v. injection of a racemic formulation of RBZ to cattle in the current trial, was markedly different. The (–)ABZSO enantiomer was depleted faster from the bloodstream compared with the (+) antipode. The (–) sulphoxide antipode is thought to be a substrate for the cytochrome P450-mediated production of the inactive sulphone metabolite (Delatour *et al.*, 1990); this may have accounted for the faster (–)ABZSO depletion from plasma, which may also confirm a substrate enantioselectivity in the production of the sulphone metabolite. Consistently, the pronounced decay of the plasma concentrations of the (–) antipode with detection up to 16 h post-treatment, correlated with a sustained increase of the $ABZSO_2$ plasma profiles that reached its peak concentration at 13 h post-treatment.

The largest quantities of ABZSO enantiomers were obtained in all the tissues and fluids analysed at 4 h post-treatment. As observed in the bloodstream, the (+)ABZSO was the enantiomeric form recovered at the highest concentrations in bile and in the fluid content and mucosa of the abomasum at all post-treatment sampling times (Table 3). The (+) antipode was also the predominant analyte recovered in lung tissue and upper intestine mucosa and fluid up to 12 h postadministration of RBZ to cattle. No marked differences between the concentration profiles of both enantiomers were observed in the liver tissue. However, in the liver tissue, as well as in the mucosa of the upper section of the small intestine, the (–) enantiomer prevailed in the latest sampling points. Although further work is required to interpret these results, an enhanced availability of the (+)ABZSO enantiomer was clearly observed in the bloodstream and in most of the parasite location tissues evaluated.

Albendazole was recovered from the liver, lung and GI mucosal tissues/fluids of RBZ-treated calves. Large quantities of

Time post-treatment	Abomasal mucosa		Abomasal fluid	
	ABZ	ABZSO	ABZ	ABZSO
4 h	12.1 (8.14–16.1)	8.07 (4.54–11.6)	0.17 (0.15–0.20)	30.0 (27.1–32.9)
12 h	1.89 (1.65–2.13)	0.39 (0.27–0.50)	0.22 (0.21–0.23)	3.54 (3.41–3.66)
20 h	0.37 (0.35–0.39)	0.03 (0.01–0.05)	0.13 (0.10–0.16)	0.33 (0.28–0.38)
28 h	0.31 (0.28–0.33)	0.17 (0.01–0.33)	0.13 (0.11–0.15)	0.44 (0.33–0.55)
32 h	0.31 (0.27–0.34)	ND	0.09 (0.09–0.09)	0.25 (0.22–0.27)

Table 4. Comparison of the relative distribution of albendazole (ABZ) and total ABZ sulphoxide (ABZSO) between abomasal mucosa and fluid, obtained after the intravenous administration of ricobendazole (7.5 mg/kg) to cattle

ND: not detected.
Values represent the mean (range values in brackets) concentrations of ABZ and total ABZSO in abomasal mucosa (µg/g) and fluid (µg/mL) obtained at different times post-treatment.

ABZ were measured in the target tissues at 4 h post i.v. administration of RBZ; the amount of that analyte recovered being particularly high in the lung (mean concentration >4 μg/g), small intestine mucosa (>7 μg/g) and abomasal mucosal tissue (>12 μg/g). Reductive metabolism of BZD sulphoxides can occur in the GI tract; ABZSO is reduced to its parent thioether (ABZ) by the ruminal/intestinal microflora of sheep and cattle (Lanusse *et al.*, 1992). A plasma-GI pH gradient and/or an active gastric secretion process, may facilitate the distribution of ABZSO from plasma to the digestive tract lumen, allowing its microflora-mediated sulphoreduction to form ABZ either in the rumen or intestine. Additionally, both ABZSO enantiomers may be secreted in saliva. It is known that drugs can be exchanged from plasma to saliva, reaching the rumen (Dobson, 1967). The plasma/saliva exchange and the large volume of saliva produced by cattle, may have contributed to the presence of ABZSO enantiomers in the rumen, where ABZ is produced by a bacteria-mediated sulphoreduction. The measurement of ABZ in different target tissues of RBZ-treated animals, is a relevant finding that may help to understand the relationship between kinetic/metabolic behaviours and efficacy of BZD methylcarbamate anthelmintics in ruminants.

The exchange of ABZ and its metabolites from plasma to different GI compartments has been previously shown (Lanusse *et al.*, 1993; Alvarez *et al.*, 1999). Ricobendazole is a weak base (pKa 7.8) and at plasma pH there will be a higher proportion of this molecule in the lipophilic nonionic form, which facilitates its passive diffusion from plasma to different tissues, including the digestive tract. An extensive ionic trapping in the abomasum has been shown after s.c. administration of RBZ to cattle (Lanusse *et al.*, 1998). The results reported here are consistent with those previous findings; enhanced concentration profiles of total ABZSO were obtained in both GI fluids compared with those measured in plasma. In addition, increased concentration levels of total ABZSO were observed in abomasal and small intestinal fluids compared with their respective mucosa (as shown in Table 4), which may be based on the higher affinity of ABZSO for the most polar medium represented by the GI fluids. Conversely, the highest ABZ concentrations were observed in both GI mucosal tissues, which is consistent with the higher lipophilicity of the drug compared with its sulphoxide derivative. Similar results were observed after the i.r. administration of ABZ to sheep, where the ratios of *AUC* abomasal mucosa/fluid were 2.58 for ABZ and 0.39 for ABZSO (Alvarez *et al.*, 2000). Altogether these results indicate a selective partitioning of ABZSO to the more polar GI fluid medium (see Table 4).

The work reported here describes the relationship between plasma kinetics and the comparative tissue concentration profiles of the two enantiomeric forms of ABZSO after administration of a racemic formulation of RBZ to cattle. An enantioselective plasma disposition and tissue distribution of ABZSO accounts for the higher availability of the (+), and the faster depletion of the (–) enantiomer, observed in this study. These findings complement those earlier reported by Delatour *et al.* (1990, 1991) on the plasma profiles of these enantiomers, and contribute to the comprehension of the impact of chiral behaviour within the pharmacology of BZD-sulphoxide anthelmintics.

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