In vivo and *ex vivo* uptake of albendazole and its sulphoxide metabolite by cestode parasites: relationship with their kinetic behaviour in sheep

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The current experiments correlate the disposition kinetics of albendazole (ABZ) following its intravenous (i.v.) and intraruminal (i.r.) administrations to *Moniezia* spp.-infected sheep, with the pattern of drug/metabolite uptake by tapeworms collected from treated animals. The *ex vivo* uptake pattern of ABZ and albendazole sulphoxide (ABZSO) by the same cestode parasite was also investigated. Naturally infected (*Moniezia* spp.) Corriedale lambs were treated with ABZ by either i.v. (Group A, $n = 15$) or i.r. (Group B, $n = 15$) administration at 7.5 mg/kg. Plasma and abomasal fluid samples were obtained over a 120-h period. Two animals per group were killed at 0.5, 1, 2, 4 and 6 h post-treatment; parasite material (tapeworms), bile and intestinal fluid samples were recovered. Furthermore, *Moniezia* spp. tapeworms obtained from sheep killed at the local abattoir were incubated with either ABZ or ABZSO for different time periods in a Kreb's Ringer Tris buffer (*ex vivo* experiments). Samples were analysed by high performance liquid chromatography for ABZ, ABZSO and albendazole sulphone ($ABZSO_2$). ABZ plasma concentrations decreased rapidly and were not detectable beyond 10 h following i.v. administration. ABZSO and $ABZSO_2$ were the metabolites recovered in plasma after both treatments. ABZ and its metabolites were extensively distributed to the digestive tract, mainly into the abomasal fluid, after the i.v. and i.r. administrations. The parent drug and its active ABZSO metabolite were recovered in tapeworms collected from both i.v. and i.r. treated lambs. However, the availability of both ABZ and ABZSO was higher in parasite material recovered from i.v. treated animals. The uptake of ABZ by the cestode parasite, both *in vivo* and *ex vivo*, was significantly greater than that of its sulphoxide metabolite, which agrees with the higher lipophilicity of the parent drug.

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INTRODUCTION

Albendazole (ABZ) is a benzimidazole methylcarbamate anthelmintic compound effective against lungworms and gastrointestinal (GI) nematodes, tapeworms and liver flukes (Campbell, 1990; McKellar & Scott, 1990). The intrinsic anthelmintic action of benzimidazole compounds on the parasite relies on a progressive disruption of basic cell functions as a result of their binding to parasite tubulin and depolimerization of microtubules (Lacey, 1990).

Albendazole is poorly soluble in water which limits its formulation to suspensions, pastes or granules; ABZ is largely used as micronized suspensions for oral and intraruminal (i.r.) administration to sheep and cattle. The acidic abomasal pH facilitates the dissolution of drug particles and subsequent absorption of the active ingredient in the lower GI tract (Hennessy, 1993; Lanusse & Prichard, 1993). Once absorbed, ABZ is extensively metabolized in the liver microsomal fraction in all the species studied (Gyurik *et al.*, 1981); the flavin-monooxygenase (FMO) (Galtier *et al.*, 1986) and cytochrome P-450 enzymatic systems (Souhaili-El Amri *et al.*, 1987) are primarily involved in ABZ biotransformation. The successive ABZ oxidations lead to more polar and less anthelmintically active metabolites. In terms of binding to parasite tubulin, ABZ parent drug is more potent than its sulphoxide metabolite (ABZSO), while the sulphone ($ABZSO_2$) is an inactive derivative (Lacey, 1990; Lubega & Prichard, 1991). The sulphoxide and sulphone metabolites dominate the plasma profile in sheep treated with both ABZ

(Marriner & Bogan, 1980; Hennessy *et al.*, 1989; Lanusse *et al.*, 1995) and netobimin pro-drug (Lanusse *et al.*, 1992), being the major analytes recovered in urine which is the main route of ABZ/metabolites elimination (Hennessy *et al.*, 1989).

Albendazole parent drug has not been detected in plasma following its enteral administration to sheep and cattle, and ABZSO has been postulated as responsible for the activity against lungworms. However, the presence of both anthelmintically active molecules, ABZ and ABZSO, in the abomasum and intestine for an extended period of time has been observed in treated sheep (Marriner & Bogan, 1980) and cattle (Lanusse *et al.*, 1993; Sánchez *et al.*, 1997), which may be of major relevance in terms of anthelmintic activity against GI parasites. It has also been shown that ABZSO may be reduced back to ABZ parent drug by the GI microflora (Lanusse *et al.*, 1992). This reductive biotransformation activity, more important in sheep than in cattle, may assure the presence of ABZ in the intestine. A reversible plasma-GI tract exchange facilitates a pH gradient-mediated concentration of ABZSO in the GI compartments, which could act as a source of ABZ. As ABZ has greater affinity for parasite tubulin than its sulphoxide metabolite this bacteria-mediated reduction may have significant importance for efficacy against GI parasites.

Plasma concentration profiles reflect those attained at the different fluid/tissues where the target parasite may be located; thus, the characterization of the plasma disposition kinetics of benzimidazole anthelmintics in ruminants has contributed to optimizing parasite control. However, the relationship between the concentration profiles of the active drug/metabolites, and the pattern of drug uptake by different target parasites needs to be elucidated. The current experiments correlate the disposition kinetics of ABZ/metabolites in plasma, GI fluids and bile following its i.v. and i.r. administration to *Moniezia* spp.-infected lambs, with the pattern of drug and metabolite uptake by tapeworms collected from treated animals. Complementary studies to characterize the *ex vivo* uptake of ABZ and its sulphoxide metabolite by cestode parasites were also carried out.

MATERIALS AND METHODS

In vivo *experiments*

Experimental animals

Thirty (30) male, 6-month old Corriedale lambs (25 ± 4.3 kg) naturally infected with *Moniezia* spp. were used in the studies described in this article. Natural infection with *Moniezia* spp. was confirmed by identification of tapeworm segments in faeces of each individual animal. The animals were fed on a high-quality alfalfa hay in quantities required to maintain stable weight over the course of the experimental period. Water was provided *ad libitum*. The experimental protocol was conducted under internationally accepted animal welfare guidelines.

Experimental design and treatments

Experimental animals were randomly divided into two balanced groups (A and B) of 15 lambs each. Animals to be used in the kinetic trial ($n = 10$) were surgically fitted with a permanent cannula in the pyloric region of the abomasum, following an adaptation of the technique described by Komarek (1981). Those animals were allowed a 4-week postsurgery recovery period before starting the kinetic experiments. Finally, the lambs in both groups were treated as follows:

Group A (i.v. treatment): animals received ABZ as a 2% (w/v) solution in propylene glycol/dimethyl sulphoxide (80: 20) (Anedra, Buenos Aires, Argentina) by intravenous (i.v.) administration into the left side jugular vein at a dose of 7.5 mg/kg.

Group B (i.r. treatment): animals received ABZ (10% micronized suspension, Valbazen^{R}, Pfizer Inc., Buenos Aires, Argentina) by intraruminal (i.r.) administration at 7.5 mg/kg.

Sampling collection

Blood (10 mL from the right side jugular vein) and abomasal digesta (15 mL, *via* cannula) samples were obtained from the cannulated animals ($n = 5$ for each experimental group), prior to and at 0.5, 1, 2, 4, 6, 8, 10, 12, 18, 24, 30, 36, 48, 60, 72 and 120 h post-treatment. Additional blood samples were taken at 2.5, 5, 10 and 15 min after the i.v. administration of ABZ. Plasma was separated by centrifugation at $2000 \times \boldsymbol{g}$ for 15 min. Abomasal fluid was recovered from abomasal digesta by centrifugation at $3000 \times \boldsymbol{g}$ for 20 min. Plasma and abomasal fluid samples were stored at –20°C until analysed by high performance liquid chromatography (HPLC).

Two animals per group were killed with a captive bolt gun and rapidly exsanguinated *via* the jugular vein at 0.5, 1, 2, 4 or 6 h post-treatment. Blood, bile, intestinal fluid samples and parasite material (*Moniezia* spp.) were recovered from each animal in both experimental groups. The tapeworms collected were rinsed extensively with saline solution and blotted on coarse filter paper and immediately processed for drug/metabolites chemical extraction as detailed below. The rest of the samples were frozen (–20°C) until analysed to determine ABZ/metabolites by HPLC. Results from this experiment (*in vivo* parasite uptake studies) are presented as drug availability obtained up to 6 h post-treatment, the last sampling collection time at which tapeworms were recovered from treated lambs; in fact, tapeworm parasites were not found in infected animals killed beyond that time post-administration.

Ex vivo *experiments*

Specimens of *Moniezia* spp. were collected from the small intestine of untreated lambs killed at the local abattoir. The parasite material was rinsed extensively with saline solution to remove intestinal debris and adhering materials. The collected tapeworms were maintained for 2 hours before starting the incubation in a Kreb's Ringer Tris (KRT) buffer (pH = 7.4) at 37°C (McCracken & Lipkowitz, 1990). A 1 g piece of parasite material (from the middle part of the strobila) was incubated at 37°C in 10 mL of the KRT buffer containing either ABZ or ABZSO at a final concentration of 3.7 μM ($\approx$ 1 μg/mL). This concentration was chosen following the available data on intestinal concentration levels for these analytes obtained in calves treated with netobimin (an ABZ pro-drug)

(Lanusse *et al.*, 1993). The following incubation times were used to evaluate the uptake of both analytes by the tapeworms: 5, 10, 15, 30, 45, 60, 90, 120 and 180 min. There were four replicate assays for each incubation time. Blank samples containing parasite material and medium without drug were incubated during the same time intervals. Once the incubation was over, the tapeworms were rinsed thoroughly with saline solution, blotted on coarse filter paper and immediately processed to extract ABZ and ABZSO.

Analytical procedures

The extraction procedures for plasma, abomasal and intestinal fluids samples were as previously described (Lanusse *et al.*, 1993). Bile samples were spiked with oxibendazole (OBZ) as internal standard (98.7% pure, 1 μg/10 μL methanol) and ABZ, ABZSO and $ABZSO_2$ were extracted using Sep Pak cartridges (Waters Associates, Milford, MA, USA) previously conditioned with 5.0 mL of 0.017 M ammonium dihydrogen phosphate (pH = 5.5). There was a solvent-mediated extraction before Sep Pak clean-up following a modification of the procedure previously described by Hennessy *et al.* (1987). Other unconjugated and conjugated ABZ metabolites present in bile were not quantified due to the lack of analytical standards.

A 1 g aliquot of parasite material (*Moniezia* spp.) was spiked with OBZ as internal standard, homogenized (Ultra-Turrax^R, T 25, Ika Works Inc., Wilmington, NC, USA) at 4°C in KRT buffer with a 1: 2 w/v tissue/solution ratio. The parasite material homogenate was mixed with 3 mL of ethyl acetate and shaken over 10 min (three times) to extract ABZ and its metabolites. The collected ethyl acetate phase was evaporated to dryness under a stream of nitrogen at 37°C. The drug/metabolites residue was dissolved in 1 mL methanol/H_2O solution (20/80) and prepared for HPLC analysis using the extraction procedure described for plasma. All the solvents and reagents (Baker Inc., Phillipsburg, NJ, USA) used during the extraction and drug analysis were HPLC grade.

Drug and metabolites analysis

Experimental and fortified plasma, abomasal/intestinal fluids, bile and parasite material samples were analysed for ABZ and its metabolites using HPLC (Shimadzu 10 A HPLC system, Kyoto, Japan) methods. Chromatographic conditions, retention times and limits of detection for ABZ and its metabolites were as previously reported (Lanusse *et al.*, 1993). The limits of quantification for ABZ and its metabolites were 0.1 and 0.05 μg/mL, respectively. Identification of ABZ, ABZSO and $ABZSO_2$ was undertaken by comparison with the retention times of pure standards (donated by Schering Plough, Kenilworth, NJ, USA), which were also used to prepare standard solutions to construct the calibration lines for each molecule in the different biological fluids and parasite material analysed. The linear regression lines for each analyte in the range of 0.01–10 μg/mL (triplicate determinations) showed correlation coefficients between 0.985 and 0.998. Recovery of ABZ/metabolites ranged between 95 and 99% (plasma), 87–95% (bile, abomasal and intestinal fluid) and 84–90% (tapeworms). Drug/metabolites concentrations were quantified by comparison of each analyte and the internal standard peak area, using Class LC 10 Software (Shimadzu, Kyoto, Japan), on an IBM 486-AT computer.

Pharmacokinetic analysis

Plasma and abomasal fluid concentration vs. time curves for ABZ and its metabolites for each individual animal were fitted with the PKCALC Computer Program (Shumaker, 1986), coupled to an augmented copy of the stripping program STRIP (Brown & Manno, 1978). The following equation was used to describe the biexponential concentration–time curves for the different analytes detected in plasma and abomasal fluid after the i.r. treatment (Notari, 1987):

$$Cp = Be^{-\beta t} - Be^{-kt}$$

where Cp = concentration at time t after administration (μg/mL); B = concentration at time zero extrapolated from the elimination phase (μg/mL); e = base of the natural logarithm; β = terminal slope (h^{-1}); and k is the rapid slope obtained by feathering which represents either the first order absorption rate constant (Kab) or first order metabolite formation rate constant (Kf)(h^{-1}). The elimination half-life ($t_{½el}$) was calculated as ln 2/β. Peak concentration (C_{max}) and the time of the peak concentration (t_{max}) were observed from the plasma or abomasal fluid concentration time curves. The area under the concentration vs. time curves (*AUC*) for plasma, bile, abomasal and intestinal fluid and parasite material samples were calculated using the trapezoidal rule (Gibaldi & Perrier, 1982). Mean residence time (*MRT*) values were calculated as *AUMC*/*AUC* (Perrier & Mayersohn, 1982), where *AUMC* is the area under the curve of the product of time and the plasma drug concentration vs. time from zero to infinity (Gibaldi & Perrier, 1982), and *AUC* is as defined above.

The data points generated for ABZ parent drug in plasma after its i.v. administration were best-fitted to a two-compartment model:

$$Cp = Ae^{-\alpha t} + Be^{-\beta t}$$

where A and B are primary and secondary disposition intercepts; α and β were the primary and secondary disposition rate constants (h^{-1}); and Cp was the plasma concentration of ABZ at time t. The distribution and elimination half-lives were calculated as ln 2 divided by the rate constants. The estimated plasma concentration of ABZ parent drug at zero time (Cp°) after its i.v. administration was the sum of the extrapolated zero-time concentrations of the coefficient A and B. Estimation of the volume of the central compartment (Dose/Cp°) and microconstants were also obtained. Total body clearance (Cl_B) was calculated by:

$$Cl_B = \text{Dose}/AUC$$

The volume of distribution (Vd_{area}) was estimated by the following equation:

$$Vd_{area} = \text{Dose}/(AUC)(ß)$$

The area under the first moment of the plasma concentration vs. time curve (*AUMC*) was used to estimate the volume of distribution at steady-state (Vd_{ss}) according to the following equation:

$$Vd_{ss} = \text{Dose}\ (AUMC/AUC^2)$$

The estimation of the kinetic variables for ABZSO and $ABZSO_2$ in plasma and ABZ, ABZSO and $ABZSO_2$ in abomasal fluid after the i.v. administration of ABZ, was undertaken following the model-independent biexponential equation described for the i.r. treatment.

Statistical analysis

The pharmacokinetic parameters and concentration data are reported as mean ± SEM. The *AUC* data obtained in drug uptake experiments were compared by Student's *t*-test. The level of significance was set at 5% ($P < 0.05$).

RESULTS

The mean (± SEM) plasma and abomasal fluid concentrations of ABZ and its sulphoxide and sulphone metabolites obtained after the i.v. administration of ABZ to sheep are shown in Fig. 1. The concentration profiles of the same molecules obtained in plasma and abomasal fluid following the i.r. treatment with ABZ are plotted in Fig. 2. ABZ plasma concentrations decreased rapidly after its i.v. administration, being undetectable beyond 10 h post-treatment. Table 1 summarizes the plasma disposition kinetics data for ABZ parent drug obtained after its i.v. administration to sheep. Both the sulphoxide and sulphone metabolites were rapidly recovered (2.5 min post-treatment) in the bloodstream after the i.v. treatment, reaching peak concentrations at 1.25 (ABZSO) and 11.5 h ($ABZSO_2$) post-treatment. ABZ parent drug was not detected in the bloodstream after its i.r. administration to sheep; the metabolites ABZSO and $ABZSO_2$ were the only analytes recovered in plasma between 0.5 and 60 h post-treatment. ABZSO total plasma availability was greater than that obtained for the sulphone derivative following both the i.v. and i.r. administrations of ABZ. The inactive $ABZSO_2$ metabolite represented the 17% (i.v.) and 30% (i.r. treatment) of the total of ABZ metabolites recovered in plasma.

High concentrations of ABZ and its metabolites were detected in abomasal fluid after the administration of the drug by both routes. Whereas the parent compound was recovered between 0.5 and either 36 (i.v.) or 60 h (i.r. treatment), the detection of both metabolites in abomasal fluid lasted up to 72 h post-treatment. ABZSO availability in abomasal fluid was significantly greater than that obtained in plasma following both the i.v. and i.r. administrations of the anthelmintic drug. The ratio *AUC* abomasal fluid/plasma for this sulphoxide metabolite was 5.14 (i.v.) and 8.06 (i.r. treatment). The comparative kinetic description for the parent drug and both metabolites in plasma and abomasal fluid is presented in Table 2 (i.v. treatment) and Table 3 (i.r. treatment).

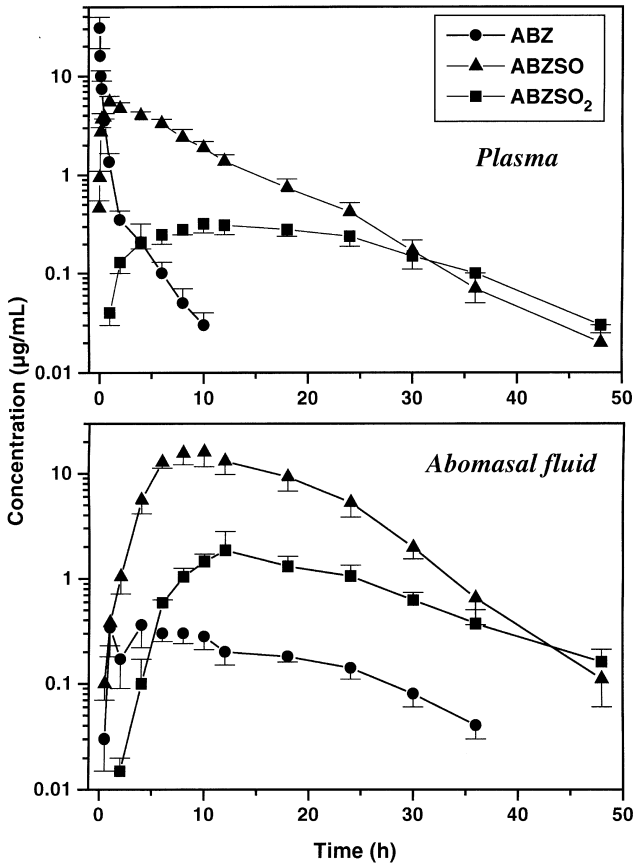

Fig. 1. Comparative (mean ± SEM) plasma and abomasal fluid concentration profiles of albendazole (ABZ), albendazole sulphoxide (ABZSO) and albendazole sulphone ($ABZSO_2$), obtained following the intravenous administration of ABZ (7.5 mg/kg) to sheep.

The concentrations of ABZ and its sulphoxide metabolite in tapeworms recovered from i.v. and i.r. treated-sheep are compared in Fig. 3. Both ABZ and its ABZSO metabolite were rapidly detected in tapeworms recovered from sheep treated by both administration routes. However, the availabilities of ABZ and its sulphoxide metabolite in the cestode parasite were greater after the i.v. compared to the i.r. treatment. Similarly, drug/metabolite availabilities in plasma and bile were greater in i.v.-treated animals than in those receiving the i.r. treatment. The comparative drug/metabolite availabilities obtained in plasma, bile, intestinal fluid and in tapeworms recovered from treated animals during the first 6 h post-treatment, expressed as *AUC* values, are shown in Table 4. The amount of ABZ parent drug recovered in tapeworms collected from treated sheep was significantly greater than that obtained for the active sulphoxide metabolite; this differential pattern of *in vivo* uptake between the parent drug and sulphoxide metabolite was observed after the administration of ABZ by both routes.

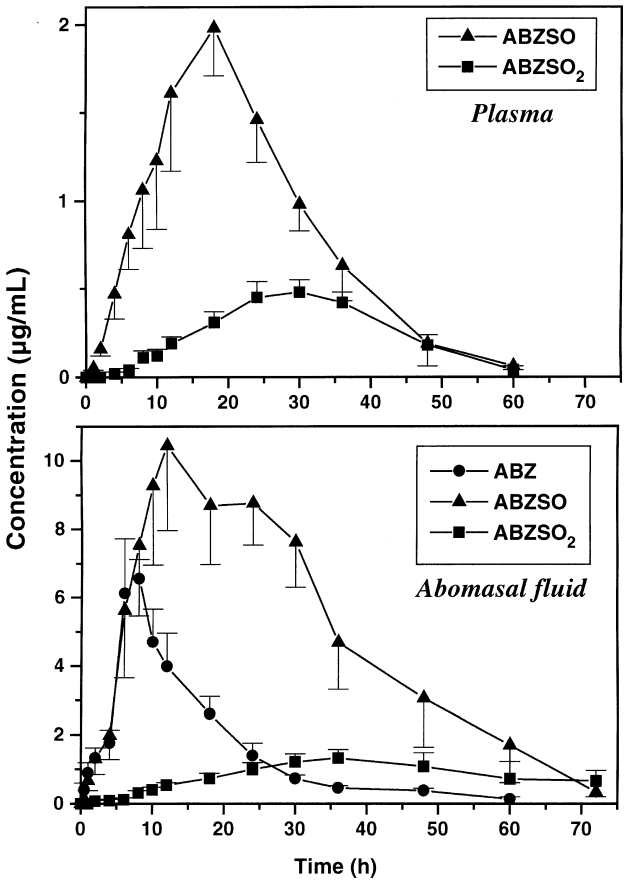

Fig. 2. Comparative (mean ± SEM) plasma and abomasal fluid concentration profiles of albendazole (ABZ), albendazole sulphoxide (ABZSO) and albendazole sulphone ($ABZSO_2$), obtained following the intraruminal administration of ABZ (7.5 mg/kg) to sheep.

Table 1. Plasma pharmacokinetic parameters (mean ± SEM) for albendazole (ABZ) obtained after its intravenous administration to sheep (7.5 mg/kg)

Pharmacokinetic Parameters	ABZ
A (µg/mL)	3.83 ± 0.38
α (h^{-1})	16.6 ± 4.20
$t_{½}\alpha$ (h)	2.00 ± 0.99
B (µg/mL)	1.13 ± 0.32
β (h^{-1})	0.67 ± 0.20
$t_{½el}$ (h)	2.07 ± 0.98
AUC (µg.h/mL)	8.28 ± 1.49
AUMC (µg.h^2/mL)	11.4 ± 3.50
MRT (h)	0.98 ± 0.25
V_c (L/kg)	0.19 ± 0.06
Vd_{area} (L/kg)	2.62 ± 0.75
Vd_{ss} (L/kg)	1.47 ± 0.49
Cl_B (L/kg.h)	1.04 ± 0.20
K_{12} (h^{-1})	8.94 ± 2.17
K_{21} (h^{-1})	1.89 ± 0.71

A, B: *Y*-axis intercepts for the phases of distribution and elimination, respectively; α, β: rate constant for the distribution and elimination, respectively; $t_{½}\alpha$: distribution half-life; $t_{½el}$: elimination half-life; $AUC_{0\text{-}\infty}$: area under the plasma concentration vs. time curve extrapolated to infinity; *AUMC*: area under the first moment curve; *MRT*: mean residence time (obtained by noncompartmental analysis of the data); V_c: apparent volume 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; K_{12}, K_{21}: compartmental rate constants.

Figure 4 shows the comparative uptake of ABZ and ABZSO by *Moniezia* spp. at the different incubation times assayed under *ex vivo* conditions. Both ABZ and ABZSO were rapidly taken up by *Moniezia* spp. after the *ex vivo* incubation, being detected in the parasite material at 5 min post-incubation. However, the uptake of ABZ at all the incubation times assayed was significantly greater than that of its sulphoxide metabolite; the total drug availability in the parasite material over the 180 min of incubation was significantly greater for the parent drug ($AUC = 8.88 \pm 0.15$ µg.h/g) compared to the more polar sulphoxide metabolite ($AUC = 2.44 \pm 0.07$ µg.h/g). The comparative availabilities of ABZ and ABZSO (expressed as *AUC* values) in *Moniezia* spp. either recovered from treated sheep (*in vivo* experiments) or obtained after the *ex vivo* incubation assays, are presented in Fig. 5.

DISCUSSION

ABZ parent drug was rapidly depleted from the bloodstream following its i.v. administration to lambs; plasma concentrations decreased rapidly and were not detectable beyond 10 h post-treatment. The metabolites ABZSO and $ABZSO_2$ appeared rapidly in the bloodstream (2.5 min) after the i.v. administration of ABZ. The rapid disappearance of ABZ and the early appearance of its sulphoxide and sulphone metabolites in plasma (i.v. administration) confirm the rapid microsomal biotransformation of the parent drug. This disposition pattern agrees with those previously reported in sheep (Galtier *et al.*, 1991) and cattle (Sánchez *et al.*, 1997) after the intravascular administration of an ABZ solution. An efficient biotransformation process and an extensive tissue distribution of ABZ may account for its rapid depletion from the bloodstream after i.v. treatment. The overall kinetic results and parasite uptake patterns observed after the i.v administration of ABZ in the current experiments, are in agreement with an extensive distribution of the parent drug and its metabolites to different tissues, including those where target parasites are located; this distribution pattern correlates with the large volume of distribution ($Vd_{area} = 2.62 \pm 0.75$ L/kg) obtained for ABZ after its i.v administration to lambs.

Consistent with kinetic data previously obtained in sheep and cattle, ABZ parent drug was not detected in the bloodstream after its i.r. administration, which has been related to a first-pass oxidation in the liver (Hennessy *et al.*, 1989; Lanusse & Prichard, 1993); however, the oxidation of ABZ to form ABZSO has been shown to occur in the enterocyte (Villaverde *et al.*, 1995) after its absorption from the small intestinal lumen, which could also account for such first-pass metabolism. Both the sulphoxide and sulphone metabolites were detected in the bloodstream for up to

Table 2. Pharmacokinetic parameters (mean ± SEM) for albendazole (ABZ), albendazole sulphoxide (ABZSO) and albendazole sulphone ($ABZSO_2$) in plasma and abomasal fluid obtained after the intravenous administration of ABZ (7.5 mg/kg) to sheep

Pharmacokinetic parameters	Plasma		Abomasal fluid		
	ABZSO	$ABZSO_2$	ABZ	ABZSO	$ABZSO_2$
C_{max} (μg/mL)	5.61 ± 0.22	0.34 ± 0.04	0.46 ± 0.08	17.2 ± 3.46	1.91 ± 0.37
t_{max} (h)	1.25 ± 0.22	11.5 ± 1.92	5.50 ± 1.30	7.50 ± 1.09	11.5 ± 0.43
$AUC_{0-\infty}$ (μg.h/mL)	50.5 ± 5.90	10.9 ± 2.15	6.02 ± 1.60	260.0 ± 49.4	40.5 ± 8.76
$t_{½el}$ (h)	5.01 ± 0.50	12.8 ± 3.83	8.51 ± 1.56	5.35 ± 0.66	10.7 ± 2.10
MRT (h)	7.58 ± 0.64	20.8 ± 4.11	11.1 ± 2.54	9.69 ± 1.09	17.3 ± 2.76
DP	2.5 min–48 h	2.5 min–36 h	0.5 h–36 h	0.5 h–72 h	2 h–48 h

C_{max}: peak concentration; t_{max}: time of the peak concentration; $AUC_{0-\infty}$: area under the concentration vs. time curve extrapolated to infinity; $t_{½el}$: elimination half-life; *MRT*: mean residence time (obtained by noncompartmental analysis of the data); DP: detection period.

Table 3. Pharmacokinetic parameters (mean ± SEM) for albendazole (ABZ), albendazole sulphoxide (ABZSO) and albendazole sulphone ($ABZSO_2$) in plasma and abomasal fluid obtained after the intraruminal administration of ABZ (7.5 mg/kg) to sheep

Pharmacokinetic parameters	Plasma		Abomasal fluid		
	ABZSO	$ABZSO_2$	ABZ	ABZSO	$ABZSO_2$
C_{max} (μg/mL)	1.92 ± 0.26	0.53 ± 0.07	7.33 ± 0.74	11.9 ± 2.04	1.49 ± 0.23
t_{max} (h)	16.0 ± 5.05	28.8 ± 2.24	7.00 ± 1.29	15.5 ± 3.50	33.0 ± 5.20
$AUC_{0-\infty}$ (μg.h/mL)	46.0 ± 5.72	19.0 ± 1.10	93.4 ± 7.50	371.0 ± 25.6	59.3 ± 15.5
$t_{½el}$ (h)	5.95 ± 1.08	9.46 ± 4.48	10.7 ± 2.49	10.6 ± 0.68	11.7 ± 2.46
MRT (h)	16.1 ± 2.03	28.4 ± 3.55	13.0 ± 2.96	17.5 ± 2.02	25.6 ± 3.36
DP	0.5 h–48 h	2 h–48 h	0.5 h–60 h	0.5 h–72 h	2 h–72 h

C_{max}: peak concentration; t_{max}: time of the peak concentration; $AUC_{0-\infty}$: area under the concentration vs. time curve extrapolated to infinity; $t_{½el}$: elimination half-life; *MRT*: mean residence time (obtained by noncompartmental analysis of the data); DP: detection period.

60 h after the i.r. treatment. As previously reported in sheep following the administration of the pro-drug netobimin (Lanusse & Prichard, 1990) and ABZ (Marriner & Bogan, 1980; Hennessy *et al.*, 1989), the active sulphoxide derivative was the main analyte recovered in plasma. This metabolic profile is different to that observed in cattle, where the sulphone is the metabolite recovered at the highest concentrations in both ABZ and netobimin-treated animals (Lanusse & Prichard, 1993). ABZSO accounted for 83% (i.v.) and 70% (i.r. treatment) of the total analytes found in sheep plasma in the current trials. On the other hand, while the parent compound was found in plasma up to 10 h post-i.v. administration, higher concentrations of its active sulphoxide metabolite were detected in the bloodstream over 48 h post-treatment; these differences in the concentration profiles between the parent drug and its active metabolite were also reflected in the GI tract, particularly in the abomasum, where ABZSO availability was significantly greater than that of ABZ parent drug after the i.v. treatment.

ABZ and its metabolites were extensively distributed from the bloodstream to the digestive tract, mainly to the abomasum, after both treatments. The rapid appearance and the large concentration profiles of the drug/metabolites found in the abomasal fluid of i.v.-treated lambs, are useful findings to demonstrate the relevance of the plasma-GI tract distribution process on the pharmacological activity of benzimidazole anthelmintics against helminth parasites established in the digestive tract. Active abomasal secretion (Hennessy, 1993) and a passive diffusion process driven by a pH plasma/abomasum gradient (Lanusse *et al.*, 1993) have been proposed as major mechanisms to explain how these molecules reach the abomasum from plasma. A marked plasma/abomasum pH gradient produces a strong ionic-trapping phenomenon, which would account for the higher concentrations of ABZSO and $ABZSO_2$ found in the abomasum compared with those attained in plasma after the administration of ABZ by both routes to sheep in the present experiments.

The availability of the sulphoxide metabolite in abomasal fluid was greater than that obtained for ABZ parent drug following both the i.v. and i.r. treatments. Similar results have been reported previously after oral/intraruminal administration of ABZ to sheep (Marriner & Bogan, 1980; Hennessy *et al.*, 1993; Alvarez *et al.*, 1997), goats (Hennessy *et al.*, 1993) and cattle (Sánchez *et al.*, 1997). An extensive adsorption of benzimidazole molecules to the particulate material of the GI digesta has been clearly described (Hennessy, 1993). Within 2 h after the i.r. administration of oxfendazole to sheep, the drug is associated with ruminal particulate digesta, and then an equilibrium between the particulate and fluid components of the digesta is established (Hennessy *et al.*, 1994). Although the parent drug and its metabolites may be adsorbed to abomasal particulate material, a lower degree of adsorption for ABZSO compared to ABZ, may account for its greater availability in the fluid phase of the abomasal content. Ali & Hennessy (1995) have demonstrated that gastric secreted oxfendazole associates less exten-

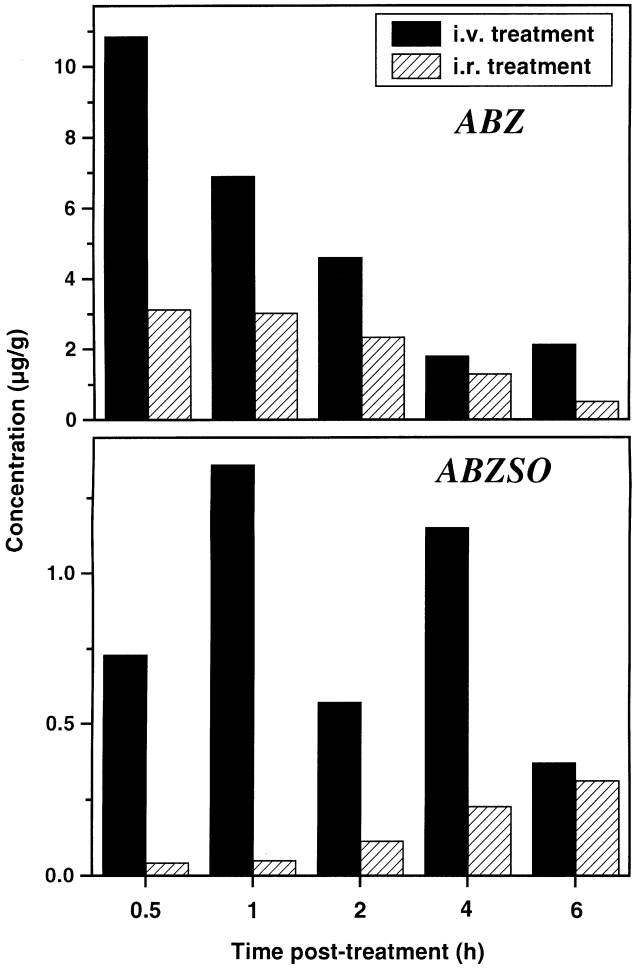

Fig. 3. Comparative concentrations of albendazole (ABZ) and albendazole sulphoxide (ABZSO) in tapeworms collected from sheep treated with ABZ (7.5 mg/kg) by intravenous (i.v.) and intraruminal (i.r.) administrations.

Table 4. Availabilities (expressed as $AUC_{0-6\ h}$) of albendazole (ABZ) and its sulphoxide metabolite (ABZSO) in plasma, bile, intestinal fluid and tapeworms collected from sheep treated intravenous (i.v.) or intraruminally (i.r.) with ABZ (7.5 mg/kg)

	ABZ		ABZSO	
	i.v.	i.r.	i.v.	i.r.
Plasma	3.10	–	15.7	2.20
Bile	12.1	6.60	96.9	5.30
Intestinal fluid	2.10	4.50	24.2	4.20
Tapeworms	19.1	9.60	4.83	1.03

Values (µg.h/mL; µg.h/g) represent drug availability expressed as mean partial *AUC*s from treatment up to 6 h postadministration. Parasite material was not found beyond 6 h post-treatment.

sively with abomasal than with ruminal particulate material, which may suggest that the acidic pH of the abomasum may play a role on the adsorption phenomenon. If the same is applicable to ABZSO, its higher concentrations in abomasal fluid

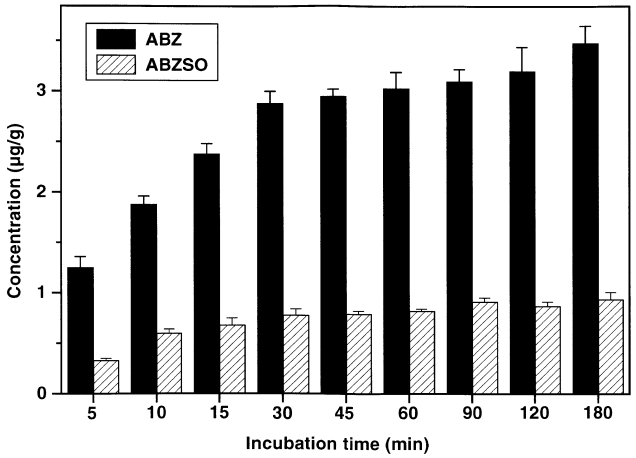

Fig. 4. Comparative *ex vivo* uptake of albendazole (ABZ) and albendazole sulphoxide (ABZSO) by tapeworms (*Moniezia* spp.). Results represent mean concentrations (± SEM) of each analyte in the parasite material after different incubation times with 4 replicates (experiments were carried out as described in Materials and Methods).

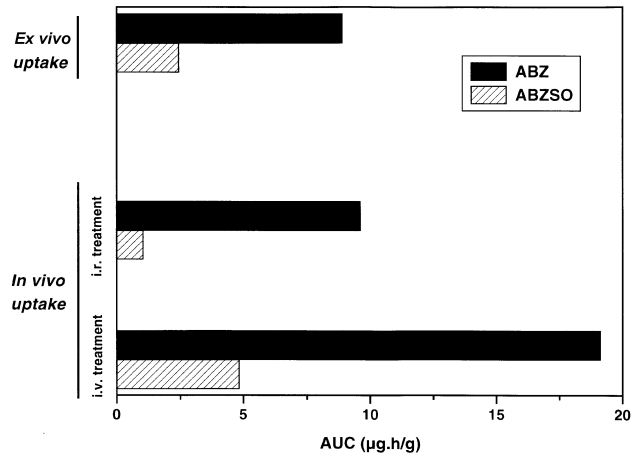

Fig. 5. Comparative uptake (expressed as *AUC*) of albendazole (ABZ) and albendazole sulphoxide (ABZSO) by cestode parasites (*Moniezia* spp.), obtained after *in vivo* and *ex vivo* experiments.

may reflect a lower degree of adsorption compared to the parent compound, which reaches the abomasum from the rumen bound to the particulate material. On the other hand, alternative explanations to the enhanced abomasal fluid availability of ABZSO compared to ABZ should be considered; the ruminal absorption of dissolved ABZ after its i.r. administration, may account for a reduced amount of ABZ reaching the abomasum. Additionally, ABZ is a source of ABZSO in the liver that could reach the abomasum from plasma as discussed above. Furthermore, the differences in lipid solubility between ABZ and ABZSO may account for a differential tissue/fluid partition coefficient; the more lipophilic parent molecule may concentrate in tissues (i.e. abomasal mucosa), while the ABZSO derivative reaches higher concentrations in the fluid portion of the digesta. Similar mechanisms could also explain the greater availability of ABZSO, compared to its parent molecule, obtained in the intestinal fluid

after the i.v. treatment. However, bile elimination of free and conjugated ABZSO could also account for its greater availability in intestinal fluid.

ABZ and its sulphoxide metabolite were detected in tapeworms recovered from sheep treated both i.v. and i.r. between 0.5 and 6 h post-treatment (Fig. 3). However, the concentration profiles of ABZ (99%) and ABZSO (300%) were significantly higher in parasite material recovered from i.v. treated animals. Plasma and bile concentration profiles for ABZ and ABZSO during the first 6 h post-treatment, were higher in i.v.-treated animals compared to those receiving the i.r. treatment (Table 4). The extensive tissue distribution of the parent drug and its metabolites correlates with the rapid detection of large concentrations of these molecules in the parasite material recovered from the gut. Cestode (*Moniezia* spp.) parasites reside in the intestinal lumen immersed in the fluid. The anthelmintically active drug/metabolites dissolved in the intestinal fluid must diffuse from the fluid into the tapeworm parasite through the external tegument. After only 0.5 h post-treatment, ABZ concentrations detected in the parasite were higher than those recovered in the intestinal fluid of the same infected lamb. This pattern was observed at all the sampling times during the first 6 h post-treatment. In fact, the availability of ABZ parent drug in the tapeworm, expressed as *AUC* values, was greater than that obtained in the intestinal fluid following the i.v. administration of ABZ. These findings confirm the rapid and efficient uptake of ABZ by the cestode parasite, which also correlates with the efficient tenicidal effect observed in this trial, where tapeworms could not be recovered from ABZ-treated lambs beyond 6 h post-treatment. Benzimidazole compounds induce marked degenerative lesions in the external tegumental surface of the cestode parasite accounting for the disappearance of the microtubular structure of the tegument (VanDen Bossche, 1986); these structural changes may account for the rapid digestion/elimination of the tapeworms from the small intestine after the ABZ treatment in the current trial.

ABZSO was the analyte recovered at the highest concentrations in the intestinal fluid of treated animals. However, in the tapeworms collected from those animals, the availability of ABZ parent drug was significantly greater than that of its sulphoxide metabolite (Fig. 5). The interaction between the cestode (*Moniezia* spp.) and its environment (intestinal fluid) occurs across the tegument (Thompson & Geary, 1995). It has been demonstrated that the transcuticular pathway is a major route of drug absorption in nematode parasites (Ho *et al.*, 1992; Sims *et al.*, 1996). Lipophilicity may also facilitate drug penetration across the nematode cuticle (Thompson *et al.*, 1989). The greater *in vivo* uptake of ABZ compared to ABZSO by tapeworms observed in the current experiments, agrees with the higher lipophilicity of the parent molecule. ABZ has an octanol/water partition coefficient of 501 compared with 14 for the more polar sulphoxide metabolite. This physicochemical difference resulting in enhanced lipophilicity, may account for the greater rate of uptake of ABZ parent drug compared to ABZSO by *Moniezia* spp. observed in the current experiments. This differential uptake pattern was confirmed by the *ex vivo* experiments, where *Moniezia* spp. cestode parasites were incubated with both molecules (Fig. 4). Similarly, the total parasite uptake for ABZ ($AUC = 8.88$ μg.h/g) was significantly greater than that obtained for the sulphoxide metabolite ($AUC = 2.44$ μg.h/g) (Fig. 4) after 180 min of incubation. ABZ and ABZSO were rapidly absorbed by the cestodes under *ex vivo* experimental conditions; both molecules were recovered from the cestode parasite after only 5 min post-incubation. ABZ concentrations gradually increased up to 180 min post-incubation, with the most marked increase observed during the first 30 min. However, ABZSO uptake reached a plateau at $\approx$ 30 min post-incubation. These results confirm the lower parasite uptake of the more polar metabolite compared with the parent drug, which correlates with the differential drug/metabolite uptake patterns observed in tapeworms collected from ABZ-treated lambs (Fig. 5).

Furthermore, ABZ parent thioether was recovered (concentrations ranging 0.2–0.3 μg/g) from cestode parasites incubated with the sulphoxide metabolite. The appearance of ABZ in parasite material incubated with the sulphoxide metabolite may indicate that the cestode parasite has the ability to biotransform ABZSO into ABZ. The presence of sulphoxide reductase activity in *Moniezia expansa* has been reported (Douch & Buchanan, 1979), which correlates with the recovery of ABZ parent drug in cestodes previously incubated with ABZSO. Both the *in vivo* and *ex vivo* studies reported here show clear evidence that ABZ parent drug concentrates in the tapeworm compared with the drug concentrations found in either intestinal fluid or incubation medium. A similar pattern has been observed in nematodes (*Ascaris suum*, *Haemonchus contortus*) in both *in vivo* and *ex vivo* studies (Alvarez *et al.* unpublished data), and in trematodes *ex vivo* (Fetterer & Rew, 1984). The high lipophilicity of the parent drug may account for its passive diffusion through the external surface of the helminth parasite. A high affinity of the drug for some lipidic tissue components of the parasite could account for the greater drug availability attained within the parasite, compared with the surrounding media.

The greater antiparasitic activity of ABZ parent drug compared to its sulphoxide metabolite has been demonstrated *in vitro*, assessing binding to parasite tubulin (Lacey, 1990; Lubega & Prichard, 1991) and nematode motility (Petersen *et al.*, 1997). The higher lipophilicity of the parent drug assures its penetration through the external parasite surface and its higher pharmacological potency in terms of tubulin affinity, which may indicate that ABZ parent drug is more relevant than its sulphoxide metabolite, in terms of anthelmintic activity against GI parasites. However, ABZSO is the most available active metabolite both in the bloodstream and in the GI tract; a reversible plasma-GI tract exchange facilities a pH gradient-mediated concentration of ABZSO in the GI compartments, which could act as a source of ABZ. The bacteria-mediated reduction of ABZSO to form ABZ in the GI tract may also contribute to achieve adequate availability of the parent drug at the site of location of target GI helminths. It is likely that the lower pharmacological potency of the sulphoxide metabolite may be compensated by its greater availability in target tissues. Therefore, the overall anthelmintic activity against

GI helminths may result from the irreversible impairment of essential microtubule-dependent cellular functions by an additive effect of both molecules.

Results of the current experiments demonstrate clearly that concentration profiles of ABZ metabolites in plasma correlate strongly with those achieved in different target tissues/fluids, which in turn, reflect the amount of drug taken up by target parasites. Thus, increased plasma availability of active drug/metabolites correlates with enhanced clinical efficacy. The simultaneous characterization of the disposition kinetics of ABZ/metabolites following the i.v. and i.r. administrations of ABZ to *Moniezia* spp.-infected sheep, with the pattern of drug/metabolite uptake by tapeworms collected from treated animals, is a relevant contribution to the understanding of the pharmacokinetic/pharmacodynamic relationship for anthelmintic drugs. The differential pattern of parasite uptake observed between ABZ parent drug and its sulphoxide metabolite, both *in vivo* and *ex vivo*, is a complement to the available knowledge of the kinetic behaviour of these drugs.

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