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Veterinary Parasitology 69 (1997) 241–253

veterinary parasitology

Modified plasma and abomasal disposition of albendazole in nematode-infected sheep

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Received 5 June 1996; accepted 13 October 1996

Abstract

The influence of gastrointestinal nematode infection on the kinetics of albendazole (ABZ) and its metabolites, albendazole sulphoxide (ABZSO) and sulphone ($ABZSO_2$) in plasma and abomasal fluid was investigated in sheep. A micronised suspension of ABZ was administered intraruminally at 7.5 $mg\,kg^{-1}$ to the following groups of sheep: (a) non-parasitised (control); (b) artificially infected with *Haemonchus contortus*; (c) naturally infected with *Haemonchus contortus* and other species of gastrointestinal nematodes. Plasma and abomasal fluid samples were obtained serially over 72 h post-treatment and they were analysed by HPLC for ABZ and its metabolites. The ABZ parent drug was not detected in plasma at any time post-treatment, however the metabolites ABZSO and $ABZSO_2$ were recovered in the bloodstream. The active metabolite ABZSO was recovered in plasma between 0.5 and 48 (uninfected), 60 (*H. contortus* infected) or 72 h (naturally infected sheep) post-administration. The area under the plasma concentration vs time curve (AUC) values for ABZSO were higher in both artificially infected (64.0 $\mu g\,h\,ml^{-1}$) and naturally infected (79.3 $\mu g\,h\,ml^{-1}$) sheep as compared with non-infected animals (41.8 $\mu g\,h\,ml^{-1}$). Peak plasma concentrations for ABZSO and $ABZSO_2$ were higher in both artificially and naturally infected sheep than in non-parasitised animals. No changes in the half-lives and mean residence times for these metabolites were observed in infected sheep. ABZ and its metabolites were found in the abomasum between 0.5 and 48 (infected animals) or 72 h (uninfected) post-treatment. The availability (total AUCs) of ABZ and its metabolites in abomasal fluid were lower in *H. contortus* infected sheep than in the uninfected control animals. The increased abomasal pH induced by the presence of the *H. contortus* infection may reduce the plasma/abomasum pH gradient, which results in a decreased ionic-trapping of ABZ and its metabolites in the abomasum. Such a phenomenon correlates with: (a) the higher total AUC values obtained for ABZ metabolites in the bloodstream of the infected compared to the control sheep,

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PII S0304-4017(96)01118-1

(b) the lower concentration profiles of the ABZ parent drug and its metabolites found in the abomasal fluid of the infected animals. © 1997 Elsevier Science B.V.

Keywords: Albendazole sulphoxide; Albendazole sulphone; Kinetics; Sheep; *Haemonchus contortus*; *Nematoda*

1. Introduction

Albendazole (ABZ) is a benzimidazole (BZD) methylcarbamate molecule which exhibits high efficacy against a broad-spectrum of helminth parasites, including lungworms, adult and larval stages of most gastrointestinal (GI) nematodes, cestodes and trematodes (Campbell, 1990; McKellar and Scott, 1990). The antiparasitic efficacy of BZD anthelmintics not only depends on their affinity for parasite β-tubulin, but also on their ability to reach high and sustained concentrations at the site of parasite location; this, in turn, depends on host-related pharmacokinetic, metabolic and tissue distribution factors (Lanusse and Prichard, 1993a). BZD anthelmintics are extensively metabolized in all mammalian species studied (Gyurik et al., 1981). ABZ parent drug is rapidly metabolized by two distinct hepatic microsomal enzymatic systems in sheep and cattle: flavin-containing monooxigenase (FMO) (Galtier et al., 1986; Lanusse et al., 1993) and cytochrome P-450 (Souhaili-El Amri et al., 1987). ABZ sulphoxide (ABZSO) (active) and ABZ sulphone ($ABZSO_2$) (inactive) are the main metabolites recovered in plasma of sheep and cattle following ABZ administration (Lanusse and Prichard, 1993b). These metabolites are extensively distributed from plasma to different tissues, including the GI tract (Lanusse et al., 1993). Since the ABZ parent drug is not recovered in the bloodstream, the active ABZSO metabolite has been postulated to be responsible for the activity against lungworms and tissue-dwelling parasites.

The presence of the parasite itself could induce important changes to pharmacokinetic behaviour, side-effects and expected efficacy of the chosen anthelmintic for therapy. Parasite-mediated inflammatory reactions with changes in mucosal permeability and in abomasal/intestinal pHs, could have an impact both on absorption and ionic-trap-mediated distribution of different BZD metabolites. During abomasal parasitism there is an elevation in abomasal pH and increased permeability of the mucosa to macromolecules (Sykes, 1978; Dakkak, 1984). The pathophysiological changes occurring during GI parasitism may affect the absorption of different drugs due to modifications in the GI transit time and atrophy of intestinal villi (Sykes, 1978). Some studies on the influence of GI parasitism on the plasma kinetics of anthelmintic drugs have been reported. Results obtained in those studies differ substantially according to the nematode species, the location and degree of the infection (parasite burden) and the anthelmintic compound under evaluation. For instance, McKellar et al. (1991) found no marked changes to the kinetics of either ivermectin, levamisole or netobimin in lambs infected with a low burden of the intestinal nematode *Nematodirus battus*. However, following artificial infection with abomasal nematodes (*Ostertagia circumcincta*, *Haemonchus contortus*), the pattern of absorption and systemic availability of fenbendazole (Marriner et al., 1985), oxfendazole (Hennessy et al., 1993) and febantel (Debackere et al., 1993) in sheep, have been shown to be altered.

The identification of factors affecting the pharmacokinetic/metabolic behaviour and efficacy of anthelmintic drugs in animals subjected to different management conditions is the goal of research in our laboratory, which may be relevant to correct and optimize treatments and to delay the development of anthelmintic resistance. The influence of both artificial and naturally acquired GI nematode infections on the plasma and abomasal disposition kinetics of ABZ and its metabolites in sheep, is reported in this article.

2. Materials and methods

2.1. Experimental design

Male Corriedale sheep (9–12 months) were used to carry out the pharmacokinetic trials reported here. Five sheep were treated with ivermectin (200 $\mu g\,kg^{-1}$) and surgically fitted with a permanent cannula in the pyloric region of the abomasum (Komarek, 1981) 6 weeks before starting the trials. These animals were housed individually and given a high quality hay and concentrate diet twice daily. Water was provided *ad libitum* to the experimental animals. After a full post-surgery recovery period the cannulated animals participated in Experiments 1 and 2.

2.1.1. Experiment 1: ABZ disposition in non-infected (control) sheep

Five cannulated animals in parasite-free conditions were treated intraruminally with a micronised suspension of ABZ (Anthelmex^R, Pfizer, Inc.) at 7.5 $mg\,kg^{-1}$. Blood and abomasal fluid samples were obtained prior to treatment and at 0.5, 1, 2, 4, 6, 8, 10, 12, 18, 24, 30, 36, 48, 60 and 72 h post-treatment. Immediately after collection, the blood samples were centrifuged at 2000 $\times g$ for 15 min and the recovered plasma was stored at -20ºC until it was analysed. After pH determination, the abomasal fluid samples were stored under the same conditions. Jugular blood samples were collected prior to the treatment for haematological determinations, including packed cell volume (PCV), total serum protein (Meites and Faulkner, 1962), albumin and globulin serum concentrations (Daumas et al., 1977).

2.1.2. Experiment 2: Influence of H. contortus *artificial infection on ABZ disposition*

After a 4-week washout period, the same five cannulated sheep in parasite-free conditions used in Experiment 1, plus two extra animals obtained from the same pool, were orally infected with 7000 L_3 of *H. contortus*. The infection was confirmed by faecal worm egg counts (EPG) at 3 weeks post-infection; EPG counts were estimated by the McMaster method (M.A.F.F., 1987). At this time, the two extra infected animals were killed by captive bolt followed by exsanguination and worm-counts were performed to confirm the presence of adult parasites and to observe the degree of macroscopic alterations in the abomasal mucosa induced by the infection. On day 28 post-infection the animals were intraruminally treated with a micronised suspension of ABZ (Anthelmex^R, Pfizer, Inc.) at 7.5 $mg\,kg^{-1}$; the sampling collection period was as described in Experiment 1. The 4-week period between infection and treatment was

allowed to permit parasite development. Blood samples were collected after 4 weeks of infection and prior to treatment to determine PCV, total serum proteins, albumin and globulin serum concentrations.

2.1.3. Experiment 3: Influence of naturally acquired GI nematode infection on ABZ disposition

Seven Corriedale sheep from the same flock, naturally infected with different species of GI nematodes (without previous treatment), were used in this experiment. Blood and faecal samples were collected for haematological and parasitological (EPG) analyses, respectively. To correlate EPG counts with the degree of nematode infection, two animals of this group were killed to perform adult worm counts. The other animals in the group ($n = 5$) were intraruminally treated with ABZ as described for Experiments 1 and 2. Blood samples were taken at different times post-treatment following the schedule described for Experiment 1. Unfortunately, it was not feasible to obtain abomasal samples from the animals in this group due to difficulties in implementing surgery procedures in the experimental animals.

2.2. Analysis of albendazole and its metabolites

All plasma and abomasal fluid samples were analysed within 6–8 weeks of their collection. Sample extraction procedures and high performance liquid chromatography (HPLC) equipment were as previously described (Lanusse and Prichard, 1990; Lanusse et al., 1993). Chromatographic conditions, retention times and limits of detection for ABZ and its metabolites were as previously reported (Lanusse et al., 1993).

Identification of ABZ, ABZSO and $ABZSO_2$ was undertaken by comparison with the retention time of pure (99.2–99.9%) reference standards (supplied by Schering Plough, Kenilworth, USA). Calibration curves for each analyte were determined. The linear regression lines for each analyte in the range 0.01–3 $\mu g\,ml^{-1}$ showed correlation coefficients between 0.985 and 0.998. Unknown concentrations of each metabolite were quantified by comparison of each analyte and the internal standard peak area, using Nelson Analytical Software, model 2600, version 3.0 (Nelson Analytical Inc., Cupertino, USA) on an IBM 386-AT computer. Spiked plasma and abomasal fluid samples were extracted and analysed by HPLC to obtain the percentages of drug recovery. The extraction efficiency of the different analytes from plasma and abomasum were 99 and 89%, respectively.

2.3. Kinetic and statistical analysis of data

The 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 ESTRIP (Brown and Manno, 1978). Pharmacokinetic parameters were determined using model-independent methods. The following equation was used to describe the biexponential concentration-time curves for the different detected analytes (Notari, 1987):

$$C_p = Be^{-\beta t} - Be^{-Kt}$$

where C_p = concentration at time t after administration ($\mu g\,ml^{-1}$); B = concentration at time zero extrapolated from the elimination phase ($\mu g\,ml^{-1}$); 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 (*Kfor*) (h^{-1}).

The elimination half-life (*Tl / 2 el*) was calculated as $\ln 2/\beta$. The peak concentration (C_{max}) and time to peak concentration (T_{max}) were read from the plotted concentration-time curve of each analyte. The area under the concentration-time curve (*AUC*) was calculated by the trapezoidal rule (Gibaldi and Perrier, 1982) and further extrapolated to infinity by dividing the last experimental plasma concentration by the terminal slope (β).

Statistical moment theory was applied to calculate the mean residence time (*MRT*) for each drug/metabolite as follows (Perrier and Mayersohn, 1982):

$$MRT = \frac{AUMC}{AUC} - \frac{1}{K}$$

where *AUC* and *K* are as defined previously and *AUMC* is the area under the curve of the product of time and the plasma drug concentration vs. time from zero to infinity (Gibaldi and Perrier, 1982).

Pharmacokinetic parameters are presented as mean ± SEM. The mean pharmacokinetic parameters obtained in plasma for each experimental group were compared by analysis of variance using the Statistical Analysis Software (SAS). Where a significant F value was obtained, Tukey's Range Test was performed to indicate order of significance. Student t-test was used to establish the level of significance between mean pharmacokinetic parameters obtained in abomasal fluid for control and *H. contortus* infected sheep (Experiment 2). In both cases a value of $P < 0.05$ was considered statistically significant.

3. Results

Faecal egg counts were 1052 (470–2890) and 2550 (1880–3380) in *H. contortus* and naturally infected sheep, respectively. The nematode species identified in the sacrificed sheep of the naturally infected group were: *H. contortus* (950), *O. circumcincta* (180), *Trichostrongylus axei* (90), *T. colubriformis* and *T. vitrinus* (1200), *Cooperia curticei* (100), *Nematodirus spathiger* and *N. fillicollis* (900) and *Trichuris ovis* (15). It is interesting to note that animals in the naturally infected group had a burden of adult *H. contortus* (950) twice the count obtained in the abomasum of the sheep artificially infected (520). There were no significant modifications to the haematological determinations between the control and both infected groups. Packed cell volume values were 34.0 ± 2.60 (control), 33.4 ± 4.80 (*H. contortus* infected) and 36.2 ± 3.13% (naturally infected). Total serum protein concentrations ranged between 5.77 and 7.23 g%. The albumin/globulin ratios were 1.06 (control), 1.12 (*H. contortus* infected) and 2.24 (naturally infected). Mean abomasal fluid pH values increased from 3.00 (control) to 3.65 (*H. contortus* infected) and 4.20 (naturally infected sheep).

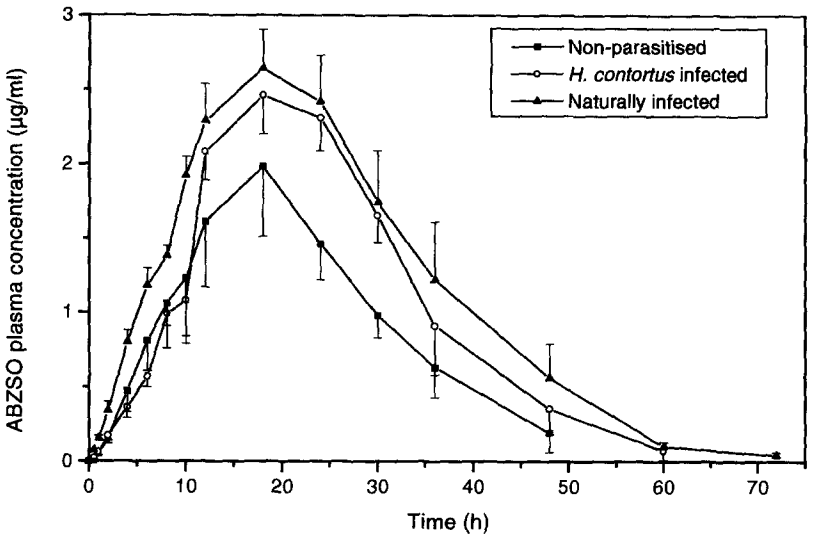

Fig. 1. Comparative mean (±SEM) plasma concentrations of ABZSO obtained following the intraruminal administration of ABZ (7.5 mg kg^{-1}) to sheep either non-parasitised, artificially infected with *H. contortus* or naturally infected with different GI nematode species.

ABZ parent drug was not found in plasma after its intraruminal administration in either group. The metabolites ABZSO and $ABZSO_2$ were the molecules recovered in plasma, ABZSO being the main analyte detected in plasma between 0.5 and either 48

Table 1
Pharmacokinetic parameters (mean ± SEM) for ABZSO and $ABZSO_2$ in plasma, obtained following the intraruminal administration of ABZ (7.5 mg kg^{-1}) to sheep either non-parasitised, artificially infected with *H. contortus* or naturally infected with different gastrointestinal nematodes

Pharmaco-kinetic parameters	Metabolites					
	ABZSO			$ABZSO_2$		
	Non-parasitised (control)	*H. contortus* infected	Naturally infected	Non-parasitised (control)	*H. contortus* infected	Naturally infected
C_{max} (µg ml^{-1})	1.76 ± 0.16	2.45 ± 0.25	2.69 ± 0.23 [a]	0.53 ± 0.07	0.69 ± 0.11	1.03 ± 0.08 [a]
T_{max} (h)	16.0 ± 5.05	20.4 ± 1.46	18.0 ± 1.69	28.8 ± 2.24	33.6 ± 1.46	26.8 ± 2.37
AUC 0–∞ (µg h ml^{-1})	41.8 ± 2.28	64.0 ± 5.76	79.3 ± 11.2 [a]	19.0 ± 1.10	21.3 ± 3.04	35.4 ± 6.22 [a]
T1/2 el (h)	5.95 ± 1.08	5.23 ± 1.42	6.62 ± 1.04	9.46 ± 1.39	7.10 ± 2.49	6.88 ± 1.00
MRT (h)	16.1 ± 2.03	17.7 ± 1.51	17.5 ± 1.56	28.4 ± 3.55	26.0 ± 2.03	25.0 ± 2.13
DP (h)	0.5–48	0.5–60	0.5–72	1–48	0.5–72	0.5–72

C_{max}, peak plasma concentration; T_{max}, time of the peak plasma concentration; *AUC* 0–∞, area under the plasma concentration vs time curve extrapolated to infinity; *T1/2 el*, elimination half-life; *MRT*, mean residence time (obtained by non-compartmental analysis of the data); *DP*, plasma detection period for ABZSO and $ABZSO_2$.

[a] Values are statistically different from those obtained for the non-parasitised group at $P < 0.05$.

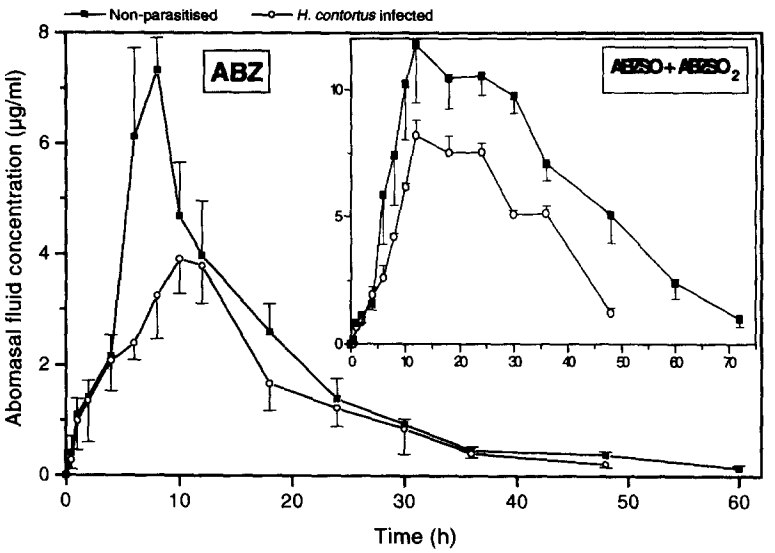

Fig. 2. Comparative mean (±SEM) abomasal fluid concentrations of ABZ parent drug obtained following its intraruminal administration (7.5 mg kg^{-1}) to sheep either non-parasitised or artificially infected with *H. contortus*. Inset: The additive abomasal concentration profiles of ABZSO and $ABZSO_2$ obtained after the same treatment are shown.

Table 2
Pharmacokinetic parameters (mean ± SEM) for ABZ, ABZSO and $ABZSO_2$ in abomasal fluid, obtained following the intraruminal administration of ABZ (7.5 mg kg^{-1}) to sheep either non-parasitised, artificially infected with *H. contortus* or naturally infected with different gastrointestinal nematodes

Pharmaco-kinetic parameters	ABZ		ABZSO		$ABZSO_2$	
	Non-parasitised (control)	*H. contortus* infected	Non-parasitised (control)	*H. contortus* infected	Non-parasitised (control)	*H. contortus* infected
C_{max} (µg ml^{-1})	7.33 ± 0.74	4.40 ± 0.76	11.9 ± 2.04	8.69 ± 0.38	1.58 ± 0.14	0.82 ± 0.1 *
T_{max} (h)	7.00 ± 1.29	11.0 ± 1.0	15.5 ± 3.50	16.5 ± 2.87	33.0 ± 5.20	33.0 ± 5.0
T1/2 el (h)	10.7 ± 2.49	10.3 ± 1.61	10.6 ± 0.68	10.5 ± 1.42	11.7 ± 2.50	9.64 ± 0.35
MRT (h)	13.0 ± 2.39	14.7 ± 1.05	17.5 ± 2.02	18.1 ± 2.86	25.6 ± 3.35	28.9 ± 5.03
Ratio *AUC* abomasum/ plasma	NA	NA	8.84	3.95 *	3.62	1.20 * *
DP (h)	0.5–60	0.5–48	0.5–72	1–48	2–72	6–48

C_{max}, peak plasma concentration; T_{max}, time of the peak plasma concentration; *T1/2 el*, elimination half-life; *MRT*, mean residence time (obtained by non-compartmental analysis of the data); *AUC*, area under the concentration vs time curve; *DP*, abomasal fluid detection period for ABZ, ABZSO and $ABZSO_2$. NA, Not applicable.

Values are statistically different from those obtained for the non-parasitised group at * $P < 0.05$ and * * $P < 0.01$.

(control), 60 (*H. contortus* infected) or 72 h (naturally infected group) post-treatment. $ABZSO_2$ accounted for 30% of the total area under the curve of the analytes detected in plasma. The comparative plasma concentration profiles of ABZSO in each experimental group are shown in Fig. 1. The results of the comparative kinetic analyses for ABZSO and $ABZSO_2$ in plasma are shown in Table 1. ABZ parent drug and its metabolites were recovered in abomasal fluid after the intraruminal treatment; the sulphoxide metabolite was also the main analyte recovered in the abomasum between 0.5 and 72 h post-treatment. The abomasal concentration profiles of ABZ and its metabolites in the different experimental groups are compared in Fig. 2. The summarised pharmacokinetic parameters obtained for all the analytes in abomasal fluid are presented in Table 2.

The plasma disposition kinetics and abomasal distribution of ABZ and its metabolites in sheep were modified by the presence of the nematode infection. The total area under the concentration vs time curves for both ABZ metabolites in plasma resulted in greater values in *H. contortus* (53%) and naturally infected sheep (90%), compared to the non-parasitised animals. However, due to individual variations in the *H. contortus* infected group, this resulted in a statistical difference only from the group with natural nematode infection. These modifications to the plasma availability of ABZ metabolites correlated with changes in the concentration profiles of these molecules in abomasal fluid. The abomasal availability of ABZ and its metabolites was lower in infected compared to non-infected animals. The comparison of the *AUC* values for the different

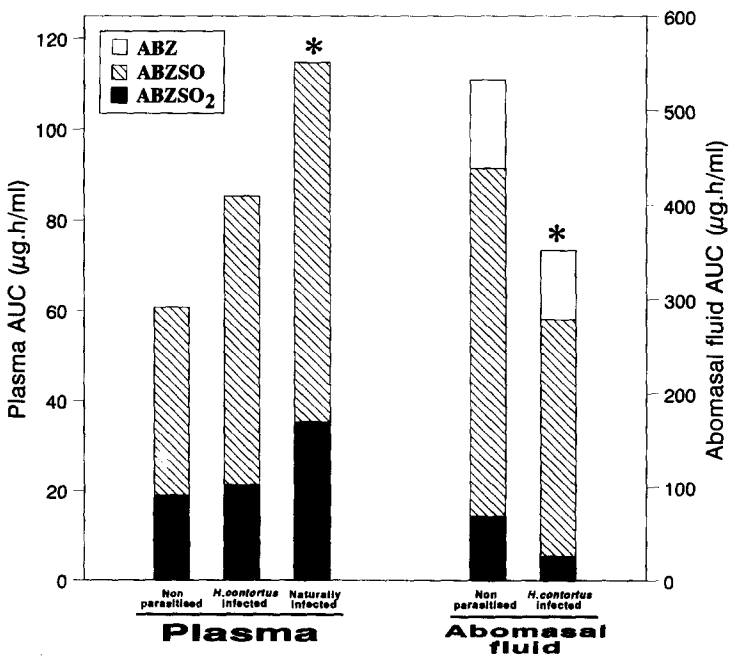

Fig. 3. Comparative total area under the concentration–time curves (*AUC*) for ABZ and its metabolites ABZSO and $ABZSO_2$ in plasma and abomasal fluid, obtained after the intraruminal administration of ABZ (7.5 $mg\,kg^{-1}$) to infected and uninfected sheep. * $P < 0.05$.

Table 3
Relationship among abomasal pH, percentage of drug ionisation and, plasma and abomasal availability of ABZ and its molecules in infected and non-infected sheep

Experimental group	Abomasal pH	Percentage of ionised drug (%) [a]	Total plasma AUC ($\mu g\,h\,ml^{-1}$)	Total abomasum AUC ($\mu g\,h\,ml^{-1}$)
Non-parasitised	3.00	100	60.8	532
H. Contortus infected	3.65	25	85.3	352 [b]
Naturally infected	4.20	6	115 [b]	ND

[a] Values estimated using the percentage of ionisation at the abomasal pH of non-parasitised animals, as a reference value (100%) of the proportion of ionic form of ABZSO. The estimation of the ionic/non-ionic form proportions was established by the Henderson–Hasselbalch equation (Baggot, 1977; based on a pKa value for ABZSO = 7.87). Total area under the concentration vs time curve (AUC) values represent the sum of the all analytes detected in plasma and abomasum, respectively.
[b] Values are statistically different from those obtained in the non-parasitised sheep at $P < 0.05$.

molecules in plasma and abomasal fluid of the different experimental groups are shown in Fig. 3. The relationship between abomasal pH values, degree of ionization and drug availability in plasma and abomasal fluid is shown in Table 3.

4. Discussion

Pathological changes occurring in both abomasal and intestinal helminth infections may affect the plasma kinetics, tissue and GI disposition of the anthelmintic drug used for therapy. However, the changes introduced to the drug pharmacokinetic behaviour depend on the severity of the infection and resultant modifications to GI physiology. The trials reported in this article investigated the influence of both artificial infection with *H. contortus* and a naturally acquired, mixed GI nematode infection, on the plasma and abomasal disposition kinetics of ABZ in sheep.

The nematode infections in animals of Experiments 2 and 3 of this trial were confirmed by the presence of eggs in faeces and the identification of adult worms in infected animals sacrificed 4 weeks post-infection. The artificial infection with *H. contortus* resulted in a mild infection, which was confirmed by: (a) the lack of clinical symptoms of parasitism; (b) the EPG counts; (c) the low parasite counts obtained in the sheep of the same infected group killed at 4 weeks post-infection; (d) the absence of modifications to the biochemical parameters measured to evaluate the pathogenic impact of the infection (packed cell volume, total serum proteins, albumin and globulin concentrations). The primary change observed in the *H. contortus*-infected sheep was the elevation of abomasal pH compared to that of the uninfected sheep. A similar pattern of infection has been reported in lambs infected with either 6600 (Kawalek and Fetterer, 1990) or 6000 L_3 of *H. contortus* (Hennessy et al., 1993). Egg counts were higher in the naturally infected animals compared to those artificially infected with *H. contortus*. *H. contortus*, *O. circumcincta*, *T. axei*, *T. colubriformis*, *T. vitrinus*, *C. curticei*, *N. spathiger*, *N. fillicollis* and *T. ovis* were the nematode species recovered from the GI tract of the naturally infected sheep. However, *H. contortus* is the most pathogenic

parasite identified in these animals. The naturally infected animals had more severe infections than those artificially infected. However, the lack of clinical symptoms and changes in haematological and biochemical parameters revealed a situation of subclinical parasitism that adequately reflects a field condition where parasite control is usually implemented.

The presence of moderate nematode infections was sufficient to identify important changes to the plasma and abomasal disposition kinetics of ABZ in both artificial and naturally infected sheep in the current trial. As previously demonstrated in sheep (Marriner and Bogan, 1980) and cattle (Prichard et al., 1985), ABZ parent drug was not found in the bloodstream. ABZSO and $ABZSO_2$ were the main analytes recovered in plasma. The *AUC* values for both ABZ metabolites in plasma were higher in infected compared to non-infected animals (Fig. 1; Table 1). ABZSO *AUC* results were between 53 (*H. contortus*) and 90% (natural infection) higher in infected sheep. A higher peak plasma concentration (C_{max}) for ABZSO was also obtained in infected animals compared to controls (Table 1). C_{max} values were between 39 (*H. contortus*) and 52% (natural infection) higher in infected sheep. Although, *AUC* and C_{max} values for ABZSO in plasma were higher in both infected groups compared to the control, these parameters did not reach statistical significance in the *H. contortus* infected group due to individual variations. The *AUC* and C_{max} values for $ABZSO_2$ were significantly higher in both infected groups compared to uninfected controls. This greater plasma availability of both ABZ metabolites in infected sheep correlated with an extended detection of both molecules in the bloodstream of those animals. An enhanced clearance of antipyrine following infection of lambs with *H. contortus* has been reported (Kawalek and Fetterer, 1990). Amongst other speculations these investigators correlated such an effect with a potential inductive response of hepatic P-450-mediated enzymatic activities triggered by the infection. This might be caused by a generalized enhancement in hepatic protein synthesis, associated with the physiological response to replace plasma proteins and other blood components lost through GI haemorrhage due to the parasite's feeding. The liver microsomal cytochrome P-450 system is involved in ABZ metabolism in sheep and cattle (Galtier et al., 1986; Lanusse et al., 1993), particularly in the oxidation of the sulphoxide to form the sulphone derivative. However, no changes in the elimination half-lives of either ABZ metabolite were observed in either the artificially or naturally infected animals in the present trial. The ratio of *AUC* ABZSO/$ABZSO_2$ has been proposed as a good indicator of the rate of liver oxidation for ABZ (Lanusse et al., 1993). There were no changes to the ratio ABZSO/$ABZSO_2$ among the different experimental groups; $ABZSO_2$ accounted for approximately 30% of the total analytes recovered in plasma. Therefore, the observed modifications to the plasma disposition kinetics of these molecules does not seem to be related to a modified pattern of liver biotransformation.

A significantly greater plasma availability of both ABZSO and $ABZSO_2$ was obtained in naturally infected compared to uninfected animals in the current trial (Figs. 1 and 3). These results do not correlate with those obtained for fenbendazole (FBZ) in *O. circumcincta* infected sheep (Marriner et al., 1985); these authors observed reduced plasma concentrations of fenbendazole metabolites in sheep. Similarly, the bioavailability of oxfendazole (OFZ) in goats (Hennessy et al., 1993) and febantel (FBT) in lambs

(Debackere et al., 1993; Landuyt et al., 1995) infected with *O. circumcincta*, was reduced compared with that of uninfected animals. The heavy burdens of *O. circumcincta* in the experimental animals of those trials may be the reason for the contradictory results on plasma availability of BZD molecules in infected animals; in contrast, the sheep treated with ABZ in the present experiments had only mild infections with either *H. contortus* or mixed GI nematode infection. However, the macroscopic lesions observed in the abomasal mucosa, the adult worm counts and the range of elevation of the abomasal pH, indicated that naturally infected animals had more severe infections than those artificially infected, which was reflected in the greater changes of the ABZ disposition occurring in those animals compared to artificially infected animals (Table 1). Some BZD absorption may occur in the rumen, but the most potent BZD compounds are poorly soluble at rumen pH. The acidic abomasal pH facilitates solubility and subsequent absorption in the lower GI tract (Lanusse and Prichard, 1993b). Gastrointestinal parasitism alters gut transit times which results in increased digesta fluid flow rates (Hennessy et al., 1993). The heavy burdens of *O. circumcincta* described in the experiments of Marriner et al. (1985) and Landuyt et al. (1995), may have shortened the GI transit time, which may have accounted for the lower absorption and plasma availability of the anthelmintic drug in infected compared to the control animals. A shorter GI transit time induced by parasitism may have a greater effect on absorption and bioavailability of compounds with low solubility in abomasal fluid, such as fenbendazole. On the other hand, the dissolution and subsequent absorption of ABZ, a compound with a relatively higher water solubility than fenbendazole (McKellar and Scott, 1990), may not have been influenced by the moderate *H. contortus* or natural infections of the animals in the trials reported here.

The ratio of non-ionized (lipophilic) to ionized (hydrophilic) forms depends upon the p*Ka* of the drug and the pH of the fluid in which the drug is dissolved, and the pH gradient between plasma and different tissues dictates the concentrations of drug/metabolite at either side of the separating cell membranes. At equilibrium, there will be a higher total concentration of the drug on the side of the membrane where the degree of ionization is greater. ABZ metabolites are reversibly exchanged between plasma and GI compartments in a distribution process driven by the plasma/GI tract pH gradient (Lanusse et al., 1993). This phenomenon facilitates a strong ionic trapping effect of ABZ metabolites at the acidic abomasal pH, which would account for the higher concentration profiles of ABZSO and $ABZSO_2$ found in the abomasum compared to plasma in all experimental groups. Parasite-mediated inflammatory reactions with changes in mucosa permeability and in abomasal pH, could have an impact on ionic trap-mediated distribution of different BZD metabolites. It has also been shown that even mild infections may exert significant pathophysiological changes in the abomasal lumen and mucosa. The abomasal *AUC* values for both anthelmintically active molecules, ABZ and its sulphoxide metabolite, were significantly higher in the control 'healthy' sheep compared to the infected animals (Figs. 2 and 3). The main modification observed in the infected sheep was the elevation of the abomasal pH. Average abomasal pH values increased from 3.0 (uninfected) to either 3.7 (*H. contortus*) or 4.2 (naturally infected animals). The increased abomasal pH, induced by the presence of the parasite, may have decreased the plasma/abomasum pH gradient and

reduced the ionic-trapping in the abomasum. Such a phenomenon would be consistent with: (a) the higher *AUC* values obtained for the ABZ metabolites in the systemic circulation of the infected compared to the control sheep (Fig. 3); (b) the lower concentration profiles of ABZ parent drug and its metabolites obtained in the abomasal fluid of the infected animals (Figs. 2 and 3). The ratio of *AUC* abomasum/plasma for ABZSO, the main active analyte recovered, was significantly lower in *H. contortus* infected sheep than in uninfected sheep (Table 2). Rather than a modified absorption pattern, these results confirm the existence of a decreased ionic-trapping of ABZ metabolites from plasma to the abomasum in infected compared to non-infected animals, which may have a relevant impact on anthelmintic therapy against GI parasites.

The quantification of drug concentrations in the abomasum not only indicates the amount of active drug at the predilection site of the most pathogenic helminths in sheep, but also reflects the pattern of active drug concentrations in the intestine. Although the intestinal nematode infections of the naturally infected animals may have contributed, the most relevant changes to ABZ disposition kinetics were provided by parasite-induced modifications to abomasal physiology. Drug pharmacokinetic characterizations have traditionally been done in healthy animals. However, the kinetic results obtained in the present trials indicated that the presence of nematode infections altered drug behaviour and that adjustment of dosage regimes may be required to optimize parasite control. This may be particularly important to control resistant strains of GI helminth parasites.

Acknowledgements

The equipment provided by Prof. Néstor Auza (Metabolism and Nutrition Research Unit) is gratefully appreciated. The authors would like to acknowledge the excellent technical assistance provided by Dr Carlos Saumell and Dr Graciela Murno. This research project was partially supported by International Foundation for Science (Sweden), Fundación Antorchas (Argentina) and Secretaría de Ciencia y Técnica, Universidad Nacional del Centro (Argentina).

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