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Dose-dependent activity of albendazole against benzimidazole-resistant nematodes in sheep: relationship between pharmacokinetics and efficacy

L. Moreno,[a,b] F. Echevarria,[c] F. Muñoz,[c] L. Alvarez,[a,b] S. Sanchez Bruni,[a,b] and C. Lanusse[a,b,*]

[a] *Laboratorio de Farmacología, Departamento de Fisiopatología, Facultad de Ciencias Veterinarias, Universidad Nacional del Centro de la Pcia. de Buenos Aires, Campus Universitario, 7000 Tandil, Argentina*
[b] *Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina*
[c] *Empresa Brasileira de Pesquisa Agropecuária, CPPSUL CP. 242, Bagé, RS 96401-970, Brazil*

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Abstract

The relationship between the pharmacokinetic behaviour and the anthelmintic efficacy of albendazole (ABZ) against benzimidazole (BZD)-resistant nematodes was studied in sheep. A micronized ABZ suspension was orally administered at two different dose levels to sheep naturally infected with BZD-resistant gastrointestinal (GI) nematodes. The experimental animals were allocated into the following groups ($n = 8$): (a) untreated control; (b) orally treated with ABZ at 3.8 mg/kg b.w.; and (c) orally treated with ABZ at 7.5 mg/kg b.w. Plasma samples were obtained serially over 72 h post-treatment from both treated groups and analysed by HPLC to measure the concentrations of ABZ and its sulphoxide (ABZSO) and sulphone ($ABZSO_2$) metabolites. Faecal egg counts were performed prior to treatment and at the necropsy day. All experimental animals were sacrificed 10 days after treatment to perform GI worm counts. While ABZ parent drug was not recovered in the bloodstream, ABZSO and $ABZSO_2$ were the molecules found in plasma. ABZSO was the metabolite measured at the highest concentrations in the bloodstream for up to 36 (treatment at 3.8 mg/kg) or 60 h (treatment at 7.5 mg/kg) post-administration. There was a proportional relationship between the administered ABZ dose and the measured plasma concentrations of both ABZ metabolites. Over a 100% increment on the plasma AUC values for the anthelmintically active ABZSO metabolite was observed at the 7.5 mg/kg compared to the 3.8 mg/kg treatment. The low efficacy patterns (<24%) observed against the GI nematodes investigated indicate a high level of resistance to ABZ given at 3.8 mg/kg an efficacious therapeutic dose rate recommended in some countries. However, the higher and prolonged plasma drug concentration measured after the 7.5 mg/kg treatment resulted in an improved efficacy pattern (estimated by both faecal egg and adult worm counts) against most of the GI nematodes studied compared to that obtained at the lower dose rate. A direct relationship between drug pharmacokinetic behaviour and anthelmintic efficacy against BZD-resistant nematodes in sheep was shown in the current work, although individual variation precluded the observation of statistically significant differences in worm counts.

Keywords: Efficacy; Pharmacokinetics; Benzimidazole anthelmintics; Albendazole; Sheep nematodes; Benzimidazole-resistant nematodes

1. Introduction

Benzimidazoles (BZD), imidazothiazoles, and avermectins/milbemycins are the most important chemical groups used to control parasitic diseases in ruminant species. The development of sheep and goat nematodes resistance to these chemical families is a seriously increasing problem worldwide (Prichard, 1990; Taylor et al., 2002; Waller, 1994). The situation in South America, is not an exception. A crucial problem was described in Brazil where about 90% of sheep farms developed resistance to albendazole (ABZ), 84% to levamisole (LEV), 73% to combination ABZ-LEV, 13% to

* Corresponding author. Fax: +54-2293-422357.
E-mail address: clanusse@vet.unicen.edu.ar (C. Lanusse).

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ivermectin, and 20% to closantel (Echevarria et al., 1996). A similar situation has been reported in Uruguay and northern Argentina (Eddi et al., 1996; Nari et al., 1996).

Studies in the UK and Europe suggest that (a) the resistance selection process occurs over a longer time frame than in Southern tropical/temperate regions and (b) for some of the key ovine nematode species little or no reversion to susceptibility may occur for many years after the withdrawal of the selecting agent (Jackson and Coop, 2000). As a consequence research on the development of vaccines and/or new drug classes, breeding for resistance, improved diagnostics, etc., have been suggested (Coles, 2001). On the other hand, the highly significant cost for the research and development of new drug compounds for food-producing animals, together with the small market share of animal health products, do not stimulate the development of new drug classes. As stated by Hennessy (1997), the chemical groups currently available in the veterinary market are all that we are likely to have in the near future and they must be used more efficiently. Understanding the kinetic behaviour of antiparasitic drugs and all the host and drug-related factors that could affect it, seems to be a pivotal issue for sustained parasite control in livestock.

The pharmacology of BZD anthelmintics in ruminant species has been broadly studied. It is well known that BZD are extensively metabolised in all mammalian species studied (Lanusse and Prichard, 1993). ABZ parent drug is rapidly metabolised by two different microsomal enzymatic systems in the liver of sheep and cattle: flavin-containing monooxigenase (FMO) (Galtier et al., 1986) and cytochrome P-450 system. ABZ sulphoxide (ABZSO) (active metabolite) and sulphone ($ABZSO_2$) (inactive metabolite) are the main molecules recovered in plasma of sheep after treatment with ABZ parent drug. It has also been demonstrated that the efficacy of BZD compounds relies on the time the parasite is exposed to ''toxic'' concentrations and that anthelmintic activity is influenced by the residence time of the drug in the animal's body (Lanusse and Prichard, 1993). Different physio-pharmacology based strategies have been assayed to increase BZD drug availability and their resultant pharmacological activity in ruminant species. Approaches such as management of feed intake (Ali and Hennessy, 1995), fasting the animals prior to treatment (Lifschitz et al., 1997; Sanchez et al., 2000), and interference with the pattern of liver biotransformation (Benchaoui and Mckellar, 1996; Lanusse et al., 1993b; Sanchez et al., 1996; Sanchez et al., 2002), have been shown to increase the systemic availability and/or residence time of active BZD molecules in the animal host.

The impact of increasing the dose rate on systemic drug concentrations and its subsequent efficacy against BZD-resistant nematodes has been extensively discussed. However, this issue remains unclear. Although, the therapeutic response to an increased BZD dose rate may depend on the genetic status of the resistant population being exposed to the drug, the results described in the current work contribute to the understanding of the issue. The rationale behind the work reported here was: (1) to evaluate the clinical efficacy of ABZ given at two different dose rates, 3.8 mg/kg (a therapeutic dose recommended against gastrointestinal nematodes in some countries), and 7.5 mg/kg against BZD-resistant nematodes in naturally infected sheep and, (2) to investigate the relationship between plasma pharmacokinetics and clinical efficacy of ABZ in infected animals.

2. Materials and methods

2.1. Animals

Twenty-four (24) crossbred F1 Corriedale–Texel sheep (26.9 kg ± 2.6), aged 9–10 months, naturally infected with BZD resistant gastrointestinal nematodes, were involved in this trial. The experimental sheep were selected from a flock where the failure of BZD compounds to control nematodes had been previously demonstrated. The selection of the animals (24) was based on worm egg per gram counts (epg). Experimental animals had an average of 916 epg ranging from 400 to 2000. On arrival at the experimental station (Bagé, Rio Grande do Sul, Brazil), animals were housed and fed with alfalfa hay and water ad libitum over a 5-days acclimation period and during the trial. On day 1 all sheep were checked for epg, ear tagged, and the individual body weights were recorded.

2.2. Experimental design, treatments, and sampling

Animals were allocated in three (3) experimental groups ($n = 8$). The allocation procedure was carried out using the ''Makegroups Software'' (Embrapa, Bagé, Brazil) which allows the random distribution of animals in the three experimental groups. The experimental animals were untreated control (Group A) and sheep orally treated with a micronized ABZ suspension (Valbazen, Pfizer, 10%) at either 3.8 mg/kg (Group B) or 7.5 mg/kg b.w (Group C). Blood samples were taken by jugular venipuncture (Groups B and C) before administration (time 0) and at 2, 4, 8, 12, 18, 24, 30, 36, 48, and 72 h post-treatment using 10 mL heparinised Vacutainers tubes (Becton–Dickinson, NJ, USA). Plasma was separated by centrifugation at 2000*g* for 15 min, placed in plastic tubes and frozen at −18 °C until analysed by high performance liquid chromatography (HPLC).

2.3. Clinical efficacy study

Worm egg counts were performed for all experimental animals prior to treatment (0 day) and at necropsy day

(10 days post-treatment). All animals were sacrificed 10 days after treatment for GI worm counts according to de World Association for the Advancement of Veterinary Parasitology (WAAVP) guidelines (Wood et al., 1995). The efficacy of each anthelmintic treatment was determined by the comparison of worm burdens in treated versus untreated animals. The following equation expresses the percent efficacy (%E) of a drug treatment against a given parasite species (S) in a single treatment group (T) when compared with an untreated control (C). The same equation was used to calculate epg reduction in the treated groups

$$\%E = \frac{\text{Mean of } S \text{ in } C - \text{ mean of } S \text{ in } T}{\text{Mean of } S \text{ in } C} \times 100. \quad (1)$$

The geometric mean was used as it most accurately represents the distribution of nematode populations within each group.

2.4. Analytical procedures

2.4.1. Plasma samples extractions

Plasma samples (1 mL) were spiked with 10 μL of oxibendazole (OBZ) (100 μg/mL) used as internal standard. ABZ and its metabolites were extracted using disposable C_{18} cartridges (Lichrolut, Merck, USA) which were previously conditioned with 0.5 mL of methanol (HPLC grade), followed by 0.5 mL of water. All samples were placed into the cartridge and then sequentially washed with 2 mL of water and eluted with 2 mL of HPLC grade methanol. Samples were concentrated to dryness in a vacuum concentrator (Speed-Vac, Savant, CE) and then reconstituted with 150 μL of mobile phase.

2.4.2. Drug quantification by HPLC analysis

Experimental and fortified plasma samples were analysed by HPLC to determine the concentration of ABZ and its metabolites. Fifty microliters of each previously extracted sample were injected in a Shimadzu 10 A HPLC System (Kyoto, Japan), using a gradient pump, UV detector set at 292 nm, an autosampler and a controller (Shimadzu Class LC10, Kyoto, Japan). A chromatographic method previously described for BZD molecules was used (Alvarez et al., 1999). Analytes were identified by the retention times of pure reference standards. Chromatographic retention times were: 3.98 (ABZSO), 6.17 ($ABZSO_2$), 8.71 (OBZ), and 10.36 min (ABZ). Calibration curves for each analyte were prepared by least squares linear regression analysis, which showed correlation coefficients between 0.995 and 0.998. The recovery of drug analytes from plasma was calculated by comparison of the peak areas from spiked plasma samples with the peak areas resulting from direct injections of standards in mobile phase. Mean absolute recoveries and coefficient of variations (CV) within the concentration range between 0.05 and 1 μg/mL (triplicate determinations) were 88.3% (CV: 5.74%) (ABZ), 86.3% (CV: 7.03%) (ABZSO), and 91.1% (CV: 3.75%) ($ABZSO_2$). Precision (intra- and inter-assay) was determined by analysing replicates of fortified plasma samples ($n = 5$) with each compound at three different concentrations (0.1, 0.5, and 1 μg/mL). Coefficient of variations ranged from 1.43 to 13.6%. The limit of detection (LOD) was estimated integrating the baseline threshold at the retention time of each compound in five spiked plasma samples. The LOD was defined as the mean 'noise'/internal standard peak area ratio plus three standard deviations (SD). The limit of quantification (LOQ) was estimated as the mean 'noise'/internal standard peak area ratio plus six SD. The estimated LOQ were 0.004 (ABZSO), 0.008 ($ABZSO_2$), and 0.009 μg/mL (ABZ).

2.5. Pharmacokinetic analysis of the data

The concentration versus time curves for ABZ and/or its metabolites in plasma for individual animals were fitted with the PKSolutions computer program (Summit Research Service, Ashland, USA). The following equation (Notari, 1987) was used to describe the biexponential concentration–time curves after the oral treatment:

$$C_p = Be^{-\beta t} - Be^{-kt}, \quad (2)$$

where C_p is the concentration in plasma at time t after administration (μg/mL); B is the concentration at time zero extrapolated from the elimination phase (μg/mL); e is the base of the natural logarithm; β is the terminal slope (h^{-1}); and k is the slope obtained by feathering which represents either the first order absorption rate constant (k_{ab}) or first-order metabolite formation rate constant (k_f) (h^{-1}). The observed 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 concentration by the terminal slope (β).

Statistical moment theory was applied to calculate the mean residence time (MRT) for metabolites in plasma as follows:

$$\text{MRT} = \frac{\text{AUMC}}{\text{AUC}}, \quad (3)$$

where AUC is 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).

2.6. Statistical analysis of the data

Pharmacokinetic parameters are presented as means ± SD. The time-based parameters (T_{max} and MRT) are

expressed as harmonic means. The Student's t test was used for the statistical comparison of the pharmacokinetic data obtained from both treatments groups. A value of $P < 0.05$ was considered statistically significant. Egg and worm counts were transformed to $\log_{10}$ (count + 1) to stabilise their variances. Analysis of variance + Tuckey test were performed for testing the main effects and interactions between parasitic species and treatments.

3. Results

ABZSO and $ABZSO_2$ were the only analytes recovered in plasma after oral treatment with ABZ at two different dosage levels in nematode infected sheep. ABZSO was the main metabolite detected in plasma for a period of 36 h (3.8 mg/kg) and 60 h (7.5 mg/kg dose treatment) post-administration. The sulphone metabolite was assessed in plasma up to 36 h post-treatment. The mean (±SD) plasma concentration profiles of ABZSO and $ABZSO_2$ obtained after oral administration of ABZ in infected sheep are shown in Fig. 1. The results of the pharmacokinetic analysis for both metabolites after both treatments are summarised in Table 1. Significantly higher peak plasma concentrations (C_{max}) and AUC values were observed for both ABZ metabolites following the treatment at the highest dose rate (7.5 mg/kg) compared to the 3.8 mg/kg treatment. Also the time of residence (MRT) of ABZSO was significantly longer after the ABZ treatment at 7.5 mg/kg. A similar trend, although without reaching statistical significance, was observed for the sulphone metabolite.

The anthelmintic efficacy obtained for both drug treatments against BZD-resistant nematodes is shown in Fig. 2 (worm counts) and Table 2 (epg counts). The low anthelmintic efficacy observed for both treatments clearly indicated that the experimental animals were infected with nematodes highly resistant to the BZD compounds. Reductions in epg at 10 days post-administration of ABZ at 3.8 and 7.5 mg/kg were 27 and 49.1%, respectively. The mean worm burdens of ABZ-resistant nematodes and their percentage of reduction after the treatments at both dosage levels are illustrated in Fig. 2. ABZ reached a 100% efficacy against *Trichostrongylus axei* at both dose rates. Only the 7.5 mg/kg

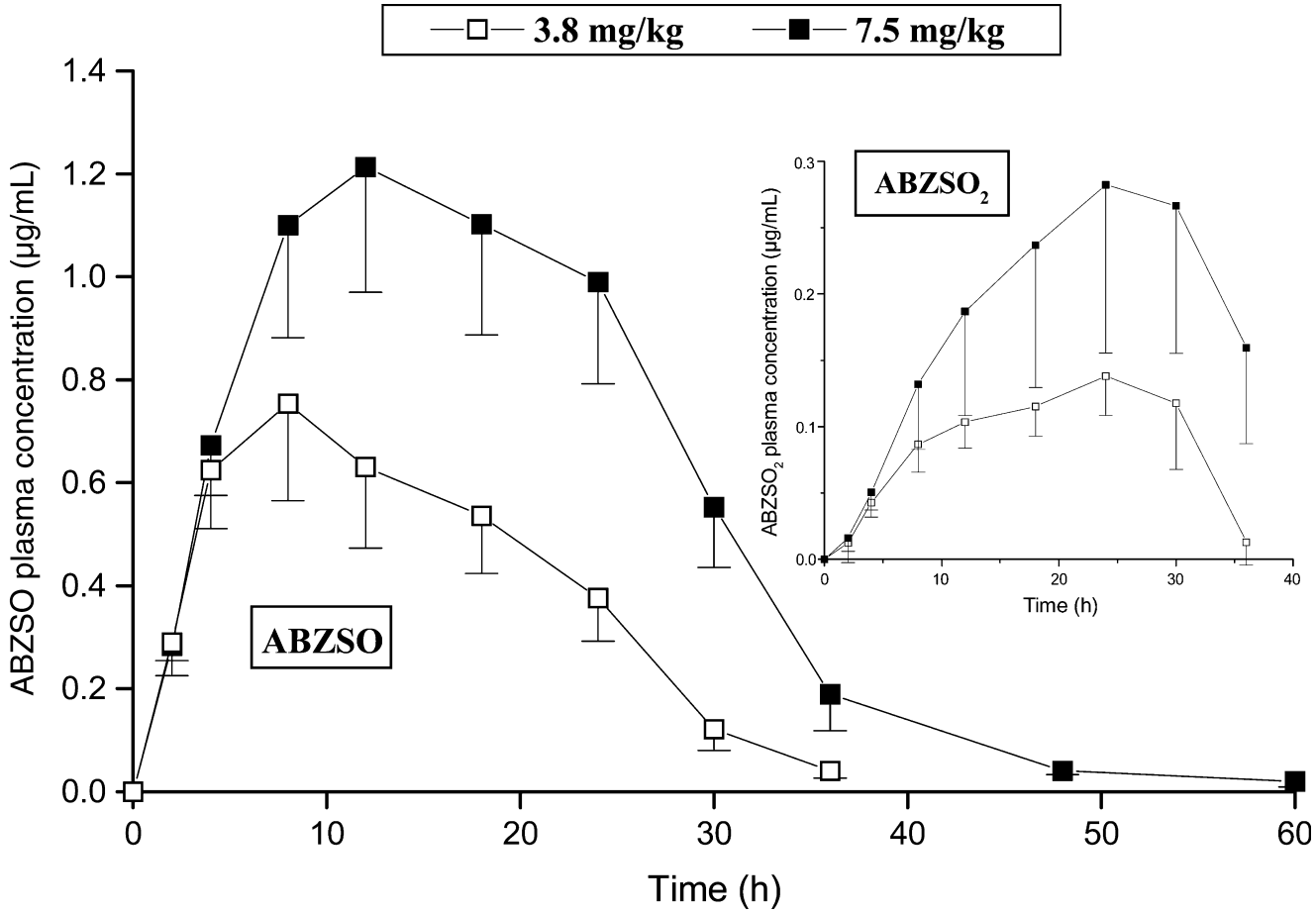

Fig. 1. Comparative mean (±SD) ($n = 8$) albendazole sulphoxide (ABZSO) and albendazole sulphone ($ABZSO_2$) plasma concentrations obtained following the oral administration of albendazole at 3.8 and 7.5 mg/kg in nematode-infected sheep.

Table 1
Pharmacokinetic (PK) parameters (mean ± SD) obtained for albendazole sulphoxide (ABZSO) and albendazole sulphone ($ABZSO_2$) after oral administration of albendazole (ABZ) at 3.8 and 7.5 mg/kg to nematode-infected sheep

PK parameters	ABZSO		$ABZSO_2$	
	Dose rate		Dose rate	
	3.8 mg/kg	7.5 mg/kg	3.8 mg/kg	7.5 mg/kg
C_{max} (μg/mL)	0.77 ± 0.17	1.23 ± 0.24**	0.13 ± 0.05	0.27 ± 0.14**
T_{max} (h)	5.82 ± 2.07	11.8 ± 2.71**	25.3 ± 2.78	23.6 ± 3.21
$AUC_{0\text{-}t}$ (μg h/mL)	14.9 ± 2.78	31.2 ± 5.43**	3.21 ± 0.75	6.65 ± 3.19**
$AUC_{0\text{-}\infty}$ (μg h/mL)	15.2 ± 2.70	31.5 ± 5.46**	3.36 ± 0.77	9.90 ± 3.68**
MRT (h)	14.9 ± 0.63	18.3 ± 0.75**	20.2 ± 2.11	29.5 ± 27.3

Values are statistically different to the 3.8 mg/kg treatment at (**) $P < 0.01$. C_{max} is the peak plasma concentration; T_{max} is the time of the peak plasma concentration; $AUC_{0\text{-}t}$ is the area under the plasma concentration vs. time curve between drug administration and 60 h post-treatment; $AUC_{0\text{-}\infty}$ is the area under the plasma concentration vs time curve extrapolated to infinity; and MRT is the mean residence time.

Table 2
Effect of albendazole (ABZ) orally administered at two different dose levels on the faecal egg output in sheep infected with benzimidazole-resistant nematodes

ABZ dose rates	Mean egg counts		Reduction (%)
	Day 0 (prior to treatment)	Day 10 (post-treatment)	
3.8 mg/kg	1843 (900–3700)	2025[a](500–3900)	27.0
7.5 mg/kg	1588 (300–3200)	1413[a](500–2800)	49.1
Untreated control	1842 (500–4100)	2775[a](200–6600)	NA

Values in brackets represent range egg counts in infected animals. Efficacy reduction (%) was calculated in comparison with the epg counts in untreated control animals at 10 days post-treatment. Epg values with the same superscripts are not statistically different ($P > 0.05$). NA: not applicable.

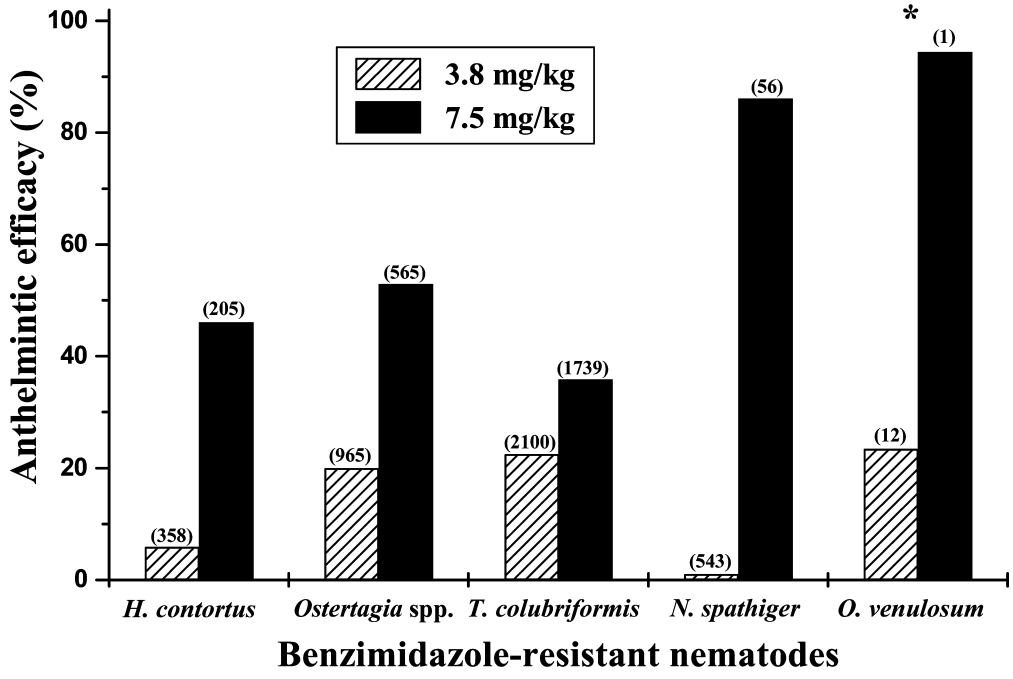

Fig. 2. Comparative albendazole (ABZ) efficacy against different benzimidazole-resistant nematodes in sheep after its oral administration at 3.8 and 7.5 mg/kg. The results indicate percentages of reduction in worm counts (at 10 days post-treatment) compared to those obtained in untreated control animals. Figures in brackets (at the top of each bar) are mean worm counts obtained in treated animals at both dose rates. *Statistically different between treatments ($P < 0.05$). The worm counts ranges at 3.8 and 7.5 mg/kg, respectively, were: *H. contortus:* (10–760), (60–450); *Ostertagia* spp: (60–1660), (270–880); *T. colubriformis:* (1130–5780), (1440–2280); *N. spathiger:* (0–1210), (0–180); *O. venulosum:* (5–31), (0–6). Mean worm burdens (including range values in brackets) in untreated control were: *H. contortus:* 380 (60–1250); *Ostertagia* spp: 1200 (180–2930); *T. colubriformis:* 2710 (980–3930); *N. spathiger*: 405 (0–1850); and *O. venulosum*: 16 (6–27).

dosage was effective and moderately effective against *Oesophagostomum venulosum* (94.4%) and *Nematodirus spathiger* (86.1%), respectively. All remaining GI nematode parasites were highly resistant to the treatment with ABZ orally administered at both dose rates. However, as it is shown in Fig. 2, the clinical efficacy against *Haemonchus contortus*, *Ostertagia* spp., and *Trichostrongylus colubriformis* tended to be higher after the administration of ABZ at 7.5 mg/kg compared to that observed at the lowest investigated dose rate. Although due to variations in individual worm counts the observed differences did not reach statistical significance, there was a clear upward trend in favour of the clinical efficacy obtained at the highest ABZ dosage. For instance, clinical efficacy against *H. contortus* increased from 5.9% (3.8 mg/kg treatment) up to 46.1% (7.5 mg/kg).

4. Discussion

As previously described, ABZ was rapidly and extensively oxidised to form the ABZSO metabolite after its oral administration to sheep (Hennessy et al., 1989; Lanusse et al., 1995). A first-pass metabolism in the liver (Hennessy et al., 1989; Lanusse and Prichard, 1993) and/or in the gut mucosa (Villaverde et al., 1995) may have accounted for the absence of ABZ parent drug in jugular blood after its oral administration at both dosage levels. ABZSO and $ABZSO_2$ were the main metabolites identified in the bloodstream. Significantly higher peak plasma concentration values (C_{max}) attained at delayed T_{max} were observed after the administration of ABZ at 7.5 mg/kg compared to the treatment at 3.8 mg/kg. The AUC of the active ABZSO metabolite, increased from 15.2 μg h/mL (treatment at 3.8 mg/kg) up to 31.5 μg h/mL (treatment at 7.5 mg/kg), which represent a 105% increment when almost a double dose was orally administered to the experimental sheep. A prolonged plasma detection period for ABZSO resulting in significantly longer MRT (22%) was observed in the animals receiving ABZ at 7.5 mg/kg. The MRT is the mean time required for an intact drug molecule to transit through the body, including absorption, metabolism, distribution, and elimination. It can be estimated for any route of administration (Martinez, 1998), so after oral/intraruminal administration the MRT would represent the residence time of the drug in the body plus the time required for absorption or metabolite formation. Regardless the administered dose rate, the MRT of a given drug should remain constant in the absence of a saturable kinetic process. Thus, the longer MRT value obtained for ABZSO after the 7.5 mg/kg compared to the 3.8 mg/kg treatment, may reflect some type of dose-dependency or non-linearity in the disposition of the active sulphoxide metabolite. Additionally, as previously reported for oxfendazole in goats (Sangster et al., 1991), the delayed T_{max} and longer MRT observed for ABZSO at the highest dose could be due to a prolonged time required for the dissolution and absorption processes, as a consequence of the larger drug suspension volume orally delivered to the animals. Some differences between treatments were also reflected in the disposition of the sulphone metabolite (Fig. 1, Table 1), whose kinetics is dependent on the metabolic formation of ABZSO in the liver.

The oral administration of ABZ under the current experimental conditions permitted the quantification of higher ABZSO plasma concentrations for longer period of time following the treatment at 7.5 mg/kg. The ABZ suspension orally administered reaches the rumen. It has been demonstrated that the rumen influences the absorption pattern and the overall pharmacokinetic behaviour of BZD compounds. An extensive adsorption of BZD molecules to the rumen particulate digesta has been shown to occur shortly after treatment (Hennessy, 1993). This extensive association between drug molecules and the particulate material of the digesta does not inhibit absorption but delays the rate of passage of the drug down the GI tract. The rumen acts as a drug reservoir and prolongs the duration of drug absorption. ABZ molecules adsorbed to ruminal particulate material gradually reach the abomasum and small intestine, the main sites of dissolution and absorption, respectively (Hennessy, 1993). Within a therapeutic dose range, when a higher oral dose is administered, a greater amount of the drug suspension will gradually pass to the abomasum being available to be absorbed at the duodenum over the time post-treatment. The lower plasma profiles of both ABZ metabolites observed at the 3.8 mg/kg treatment may be due to a lower total amount of ABZ available to be absorbed, which explains the positive relationship between the amount of drug administered and the systemically available concentrations of both metabolites in the treated animals. Furthermore, a high correlation between the concentration profiles of ABZ metabolites measured in the bloodstream and those recovered in tissues of parasite location and within target parasites collected from treated sheep, has been demonstrated (Alvarez et al., 1999, 2000). These previous pharmacokinetic findings clearly indicate that the enhanced drug concentrations and the prolonged plasma detection of the drug observed with the higher dose treatment in the current trial, may account for greater drug exposure of the target nematodes which would also explain the tendency observed in the anthelmintic efficacy against resistant parasites reported here.

The active sulphoxide metabolite (ABZSO) can be reduced back to ABZ by the ruminal and intestinal microflora (Lanusse et al., 1993a), which may contribute as an "extra" source of ABZ in the GI tract. This metabolic sulphoreduction may be of primary importance for the antiparasitic efficacy of BZD thioethers

and it has been shown to occur as a GI bacteria-mediated metabolic process. Additionally, binding to parasite tubulin is the putative mode of action for BZD compounds (Lacey, 1990) and ABZ has a greater affinity for nematode tubulin than ABZSO (Lubega and Prichard, 1991), which indicates that the bacteria-mediated reduction of the sulphoxide metabolite may have a significant importance for efficacy against GI parasites. The higher ABZSO plasma profiles obtained after ABZ administration at 7.5 mg/kg determines that a greater amount of ABZSO is available to be distributed from the bloodstream to the digestive tract, which would account for a greater formation of ABZ via reduction of the sulphoxide metabolite. This may also account for the increased final efficacy observed at the highest dose level against BZD-resistant nematodes in the current trial.

The efficacy and spectrum of activity of the BZD compounds are determined by the time of parasite exposure to toxic drug/metabolites concentrations. It has also been shown that concentration profiles of ABZ metabolites in plasma strongly correlate with those achieved in different target tissues/fluids, which in turns, reflect the amount of drug reaching target parasites. Therefore, increased plasma availability of active drug/metabolites correlates with the drug concentration that reaches the target parasite (Alvarez et al., 1999). The differences observed between ABZ dosage levels in sheep in the current work was reflected in the pharmacokinetic behaviour, which may have important implications in clinical efficacy. The administration of the highest ABZ dose rate resulted in a proportional enhancement of the C_{max} and AUC values, kinetic parameters directly related to the administered dose. This is evident considering the dose ratio ($7.5/3.8 = 1.97$) and parameter ratios for C_{max} ($1.23/0.77 = 1.59$) and AUC ($31.2/14.9 = 2.09$) for ABZSO and C_{max} ($0.27/0.13 = 2.08$) and AUC ($6.65/3.21 = 2.07$) for $ABZSO_2$, where all values are $\cong 2$, except C_{max} for ABZSO probably due to higher deviation of the data.

The reduction of the mean faecal egg output measured at 10 days post-treatment tend to be higher after the treatment at 7.5 mg/kg compared to that obtained at 3.8 mg/kg, although individual variation precluded to reach statistical significance. The low nematodicidal activity observed in this trial (<24% efficacy at 3.8 mg/kg) compared to those widely reported in the literature for ABZ and related compounds, confirms that the animals were naturally infected with nematodes highly resistant to BZD compounds. The WAAVP guidelines establish that the efficacy of a drug should be expressed for each genus/species (larvae/adults) as highly effective (over 98%), effective (90–98%), moderately effective (80–89%) or insufficiently active (less than 80%) (Wood et al., 1995). According to this guideline, ABZ was highly effective only against *T. axei* at both, 3.8 and 7.5 mg/kg dosage levels. The treatment with ABZ was also effective against *O. venulosum*, moderately effective against *N. spatiger* at 7.5 mg/kg, and insufficiently active at 3.8 mg/kg against this nematodes species. These differences in efficacy between treatments were statistically significant at $P < 0.05$ for *O. venulosum*. However, ABZ activity against *H. contortus*, *Ostertagia* spp., and *T. colubriformis* was insufficient at both dosage levels. The low efficacy patterns observed against these GI nematodes indicates a high level of resistance to ABZ given at the 3.8 mg/kg, a nematodicidal therapeutic dose recommended for ruminants in several countries. The enhanced drug concentration measured in the bloodstream after the 7.5 mg/kg treatment was consistent with the tendency towards an improved efficacy (estimated by both faecal egg and adult worm counts) against those nematodes compared to that obtained at the lowest dose rate.

From the data reported here, it can be concluded that an enhancement in plasma concentrations of the active drug/metabolite correlated with increased efficacy against some BZD-resistant nematodes species in sheep. Although fluctuation in some worm burden counts did not permit to obtain statistically significant differences between treatments, a general upward trend in the efficacy against most resistant nematodes was observed when the dose rate used increased in a 100%. These results are a further evidence that increased drug systemic availability correlates with enhanced clinical anthelmintic efficacy. Given the widespread development of anthelmintic resistance in sheep and goats nematodes, a rational use of BZD compound based on the knowledge of their pharmacological features integrated with management strategies, seems to be the only feasible approach to maintain, at least partially, their therapeutic potential.

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