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Dose-dependent systemic exposure of albendazole metabolites in lambs

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The gastrointestinal absorption of most drugs follows a first-order kinetics, whereby a constant fraction of the total drug is absorbed in each equal time interval. Although this related absorption principle is applicable to the most of the therapeutically used drugs, it remains unclear for poorly water-soluble compounds such as the benzimidazole anthelmintics in ruminants. The goal of the current work was to characterize the albendazole (ABZ) metabolites plasma disposition kinetics after ABZ administration at different dosages to nematode-infected lambs. Eighteen Corriedale lambs artificially infected with a resistant *Haemonchus contortus* strain were allocated into three groups and intraruminally treated with ABZ at either five (ABZ_5), 15 (ABZ_{15}) or 45 (ABZ_{45}) mg/kg. Blood samples were collected up to 120 h post-treatment, and the collected plasma was analysed by high-performance liquid chromatography. The estimated pharmacokinetic parameters were statistically compared using parametric and nonparametric tests. None of the animals involved in the current trial showed any adverse events during the study. While ABZ parent drug was not recovered in the bloodstream, the area under the concentration vs time curve (AUC) of the active ABZ-sulphoxide (ABZSO) metabolite increased significantly ($P < 0.05$) from 21.0 (ABZ_5) up to 158.6 (ABZ_{15}) and 389.7 μg·h/mL (ABZ_{45}), which indicates some type of nonproportionality in the relationship between dose level and drug systemic exposure. The overall kinetic disposition of the inactive sulphone metabolite did not change after treatment at threefold the therapeutic ABZ dosage. However, significantly ($P < 0.05$) higher AUC, C_{max} and mean residence time values were observed after the administration of the highest dosage level. The higher dosages accounted for a significantly ($P < 0.05$) enhancement of the ABZSO peak plasma concentration, which were obtained at delayed times post-treatment. High correlations between $AUC_{0\text{-}LOQ}$ and C_{max} and nematode counts were observed, with Spearman's coefficients of −0.83 and −0.84, respectively. The results obtained in the current experiment show that increasing the dose of ABZ in sheep is clearly associated with enhanced plasma ABZ metabolites exposure. The data showed a nonproportionality on the gastrointestinal absorption of ABZ in nematode-infected lambs.

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INTRODUCTION

Albendazole (ABZ) is a benzimidazole (BZD) methylcarbamate anthelmintic compound effective against lungworms and gastrointestinal (GI) nematodes, tapeworms and liver flukes (Campbell, 1990; McKellar & Scott, 1990). Owing to its poor water solubility, ABZ is largely used as micronized suspensions for oral and intraruminal 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). ABZSO and $ABZSO_2$ are the main metabolites found in the bloodstream after ABZ administration to sheep (Marriner & Bogan, 1980; Hennessy *et al.*, 1989; Lanusse *et al.*, 1995). The successive ABZ oxidations lead to more polar and less anthelmintically active metabolites. In terms of binding to parasite tubulin (its putative mechanism of action), 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 oral absorption of most drugs follows first-order kinetics whereby a constant fraction of the total drug present is absorbed in each equal interval of time (Neubig, 1990). This statement, true for most of the drugs commonly used in veterinary therapeutics, remains unclear for the BZD compounds in ruminant species. Zero-order absorption kinetics has been described after oral administration of fenbendazole (FBZ) to dogs at doses of 20 and 100 mg/kg (McKellar *et al.*, 1990) or 2.5–100 mg/kg (McKellar *et al.*, 1993). However, Moreno *et al.* (2004) reported a linear dose proportionality on ABZ absorption after the administration of 3.8 and 7.5 mg/kg to sheep. This apparent difference on the drug absorption pattern between animal species may be attributed to anatomical and physiological differences, where the ruminants may take the advantage to the relatively high volume of the rumen/reticulum and the resultant slower GI content transit time, compared to that observed in monogastric species.

The therapeutic response to an increased BZD dosage may depend on the genetic status of the resistant population being exposed to the drug. The impact of large dose increasing on BZD anthelmintics systemic concentrations and on the subsequent efficacy against BZD-resistant nematodes in ruminants remains unclear. The work reported here was addressed to evaluate pharmacokinetic behaviour, including the absorption pattern and disposition kinetics of ABZ metabolites following ABZ administration at different increasing dosage levels to *Haemonchus contortus*-infected lambs. The work reported here is complementary to a clinical efficacy trial addressed to characterize the clinical efficacy of the same increased ABZ doses in lambs parasitized with BZD-resistant *H. contortus*, where the genetic status of the parasite population surviving treatments at the different dose levels was established (V. Barrère et al., submitted).

MATERIAL AND METHODS

Animals

Eighteen Corriedale lambs (6–7 months old, 34.3 ± 6.3 kg), artificially infected with an *H. contortus* strain (10 000 L_3/animal) resistant to BZD anthelmintics (trial day 41), were involved in the current trial. The *H. contortus* strain was isolated from a farm where failure of ABZ to control this nematode parasite was previously demonstrated (Entrocasso *et al.*, 2008). Forty days after infection (trial day 1), all lambs were checked for faecal egg counts (epg), ear tagged and the individual body weights were recorded. Experimental animals had an average of 6155 ± 3469 epg counts ranging from 1760 to 12 060. Animals were housed in a stall without access to grass and feed with total mixed grain-based ration (Ovino®; TandilCoop, Tandil, Argentina) during the experiment and for 20 days before nematode infection. All the animals had free access to water. Animal procedures and management protocols were approved by the Ethics Committee according to the Animal Welfare Policy (act 087/02) of the Faculty of Veterinary Medicine, Universidad Nacional del Centro de la Provincia de Buenos Aires (UNCPBA), Tandil, Argentina (http://www.vet.unicen.edu.ar).

Chemicals

Pure (≥99%) standards of ABZ, ABZ-sulphoxide (ABZSO), ABZ-sulphone ($ABZSO_2$), and oxibendazole (OBZ) used as internal standard (IS), were used in the present experiment. The commercial formulation of ABZ (Valbazen®, 10% suspension) was from Pfizer Animal Health, Buenos Aires, Argentina. All the solvents (acetonitrile and methanol) used during the extraction and drug analysis were of high-performance liquid chromatography (HPLC) grade and purchased from Baker Inc. (Phillipsburg, NJ, USA). Water was double distilled and deionized using a water purification system (Simplicity®; Millipore, Brazil). Buffer salts ($ClNH_4$) were purchased from Baker Inc. (Phillipsburg, NJ, USA).

Experimental design, treatments and sampling

All lambs were randomly allocated into three experimental groups ($n = 6$). Experimental animals received the following treatments (trial day 0): ABZ_5, animals were treated with ABZ by the intraruminal (i.r.) route at the therapeutic dose of 5 mg/kg; ABZ_{15}: ABZ was administered by the i.r. route at 15 mg/kg dosage (dose × 3) and ABZ_{45}: ABZ was administered by the i.r. route at 45 mg/kg dosage (dose × 9).

Blood samples (5 mL) were taken from the jugular vein using 10 mL heparinized Vacutainers® tubes (Becton Dickinson, Franklin Lakes, NJ, USA), before administration (time 0) and at 1, 3, 6, 9, 12, 18, 24, 30, 36, 48, 54, 72, 96 and 120 h following the i.r. treatments. Plasma was separated by centrifugation at 2000 ***g*** for 15 min, placed into plastic tubes and frozen at −20 °C until analysis by HPLC. Any unusual behaviour, such as diminishing food consumption, ataxia or prostration, was recorded as a potential sign of toxic effects induced by treatment.

Analytical procedures

ABZ/metabolites analysis. Sample clean up: ABZ, ABZSO and $ABZSO_2$ were extracted using disposable C_{18} columns (RP-18, 100 mg, Strata®, Phenomenex, Torrance, CA, USA). Ten microlitres of OBZ (50 μg/mL) was added to 500 μL of plasma in a glass test tube. Spiked samples were placed into a C_{18} column (preconditioned with 0.5 mL of methanol followed by

0.5 mL water) in a vacuum system (Lichrolut®; Merck, Germany). Samples were washed (2 mL of water) and then eluted with 2 mL of HPLC-grade methanol. After elution, all samples were concentrated to dryness in a vacuum concentrator (Thermo Speed-Vac®, Milford, MA, USA) and then reconstituted with 300 μL of mobile phase.

HPLC analysis: Experimental and spiked plasma samples (used for validation) were analysed by HPLC (Shimadzu 10 A-HPLC System, Kyoto, Japan) with a UV detector set at 292 nm. Fifty microlitres of each previously extracted sample was injected, and the analytes were eluted (flow 1.2 mL/min) from the analytical column (5 μm, 250 × 4.6 mm, C_{18} column; Phenomenex Selectosil®, Torrance, CA, USA) by a binary gradient previously described (Alvarez *et al.*, 1999). The compounds were identified by the retention times of pure reference standards. Retention times for ABZSO, $ABZSO_2$, OBZ and ABZ were 4.20, 6.40, 8.80 and 10.30 min, respectively. There was no interference of endogenous compounds in the chromatographic determinations. The linearity was tested by constructing calibration curves for each compound. The peak area ratio between the molecule under study and the IS was determined for each drug to prepare the plasma calibration curves ranging from 0.01 to 15 μg/mL using triplicate analysis (n = 3). The curves were constructed by least squares linear regression analysis, giving a correlation coefficient (r) between 0.9989 and 0.9996. Mean absolute recovery percentages for concentrations ranging between 0.25 and 5 μg/mL (n = 6) were 78.0 (ABZSO), 83.3 ($ABZSO_2$), 87.5 (OBZ) and 85.2% (ABZ) with coefficient of variation (CV) of 5.5%, 2.8%, 5.2% and 7.2%, respectively. The precision of the method (intra- and interassay) was determined by analysing plasma samples (n = 6) fortified with ABZ and metabolites at three different concentrations (0.25, 1 and 5 μg/mL). The CV for the intra and interassay precision ranged from 4.70% to 7.22%. Accuracy of the method, estimated as the interday differences between observed and calculated concentration values (six consecutive working days) and expressed as the relative error (% RE), was 0.3, 11 and −3.8 for ABZSO, $ABZSO_2$ and ABZ, respectively. The limit of detection (LOD) was estimated according to the following equation (Snyder *et al.*, 1997): LOD = A/B + (SD*3) where A is the baseline threshold at the retention time of each compound (n = 6) in spiked plasma samples, B is the peak area of the IS and SD is the standard deviation obtained from A. The LOD estimated was 0.003, 0.002 and 0.002 μg/mL for ABZSO, $ABZSO_2$ and ABZ, respectively. The limit of quantification (LOQ) was defined as the lowest measured concentration with a CV <20%, an accuracy of ±20% and an absolute recovery ≥70%. The LOQ estimated for the three molecules assayed was 0.01 μg/mL. Values below LOQ were not included in the pharmacokinetic analysis.

Pharmacokinetic analysis of the data: The concentration versus time curves for ABZ metabolites in plasma for individual animals were fitted with the PKSolutions™ computer software (Summit Research Service, Ashland, OH, USA). Pharmacokinetic analysis of the experimental data was performed by noncompartmental analysis. The elimination (T½el) half-life was calculated as ln 2/β, where ß represents the terminal slope (per hour). 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 from 0 to the quantification time ($AUC_{0\text{-}LOQ}$) was calculated by the trapezoidal rule (Gibaldi & Perrier, 1982) and further extrapolated to infinity ($AUC_{0\text{-}\infty}$) by dividing the last experimental concentration by the terminal slope (β). $AUC_{0\text{-}LOQ}$ and C_{max} values were dose-normalized dividing the observed value by 3 (ABZ_{15}) or 9 (ABZ_{45}). Statistical moment theory was applied to calculate the mean residence time (MRT) for metabolites in plasma, as follows: MRT = AUMC/AUC where AUC is as defined previously and area under the first moment curve (AUMC) is the area under the curve of the product of time and the plasma drug concentration versus time from zero to infinity (Gibaldi & Perrier, 1982).

As previously mentioned, a complementary clinical study tested the efficacy against re[illegible]t *H. contortus* in the different ABZ-treated groups (V. Barrère et al., submitted). From these results, we include in the present experimental work the nematode counts of each animal, and its relationship with two (AUC and C_{max}) different pharmacokinetic parameters, strongly associated with the anthelmintic effect. Direct adult nematode counts of animals from untreated control and ABZ-treated groups (ABZ_5, ABZ_{15} and ABZ_{45}) were determined 5 days after treatment (trial day 5) following the necropsy procedures described in the World Association for the Advancement of Veterinary Parasitology (WAAVP) guidelines (Wood *et al.*, 1995).

Statistical analysis of the data

The pharmacokinetic parameters and concentration data are reported as arithmetic mean ± SD. Parametric (ANOVA + Tukey) and nonparametric (Kruskal–Wallis) tests were used for the statistical comparison of the pharmacokinetic data obtained from the different experimental groups. A value of $P < 0.05$ was considered statistically significant. Dose proportionality was indirectly determined by nonparametric analysis of the non-transformed dose-normalized ABZSO $AUC_{0\text{-}LOQ}$ and C_{max}, using the Kruskal–Wallis statistical test. Correlations between either $AUC_{0\text{-}LOQ}$ or C_{max} values with the adult *H. contortus* counts in each animal were performed by nonparametric analysis (Spearman's r). The statistical analysis was performed using the Instat 3.0 Software (Graph Pad Software, San Diego, CA, USA).

RESULTS

None of the animals involved in the current trial showed any adverse events during the study. The mean (±SD) ABZSO plasma concentration profiles after the i.r. administration of ABZ at different dosages are shown in Fig. 1a. ABZSO and $ABZSO_2$ were the main analytes recovered in plasma after ABZ treatment. The parent drug was only quantified in plasma in one animal from

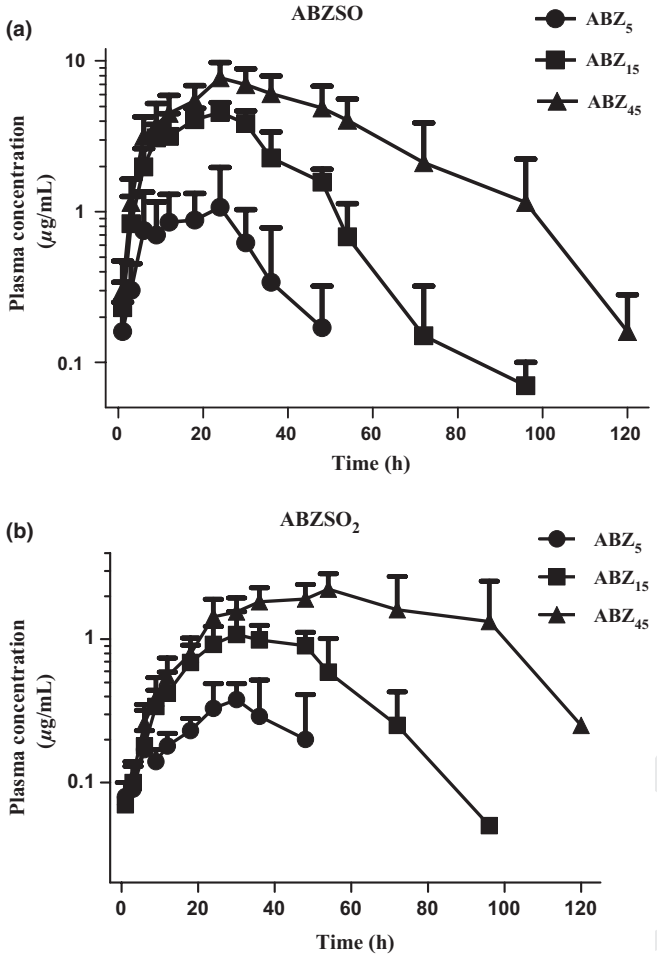

Fig. 1. Comparative mean (±SD) plasma concentration profiles (n = 6) for (a) albendazole sulphoxide (ABZSO) and (b) albendazole sulphone ($ABZSO_2$), obtained after the administration of albendazole (ABZ) by the intraruminal route at either five (ABZ_5), 15 (ABZ_{15}) or 45 (ABZ_{45}) mg/kg to *Haemonchus contortus*-infected lambs.

the ABZ_{45} group, in concentrations ranging between 0.05 and 0.06 μg/mL at 24–36 h post-treatment. The dose level affected the plasma disposition kinetics of ABZSO in treated lambs. The time of ABZSO detection in plasma increased from 1–48 h (ABZ_5) to 1–96 h (ABZ_{15}) and 1–120 h (ABZ_{45}). Table 1 summarizes the plasma pharmacokinetic parameters for ABZSO obtained after the i.r. administration of ABZ at different dosages to infected lambs. The $AUC_{0\text{-}LOQ}$ represented ≥90% of the $AUC_{0\text{-}\infty}$ for the three different experimental groups, confirming that 120 h was an adequate sampling period for the estimation of plasma disposition kinetics of ABZ metabolites in lambs. The AUC of the active ABZSO metabolite increased from 21.0 ± 6.4 μg·h/mL (ABZ_5) up to 158.6 ± 19.4 μg·h/mL (ABZ_{15}) and 389.7 ± 98.2 μg·h/mL (ABZ_{45}). Furthermore, when the mean $AUC_{0\text{-}LOQ}$ values were adjusted by the dosages, the ABZSO plasma exposure after the administration of 15 and 45 mg/kg doses was 78% and 43% higher, compared to the therapeutic dose (5 mg/kg). These AUC values show a lack of proportionality in the relationship between dose and systemic exposure, with AUC values increasing more than it was expected. The highest dose level was also correlated with a significant ($P < 0.05$) enhancement of the ABZSO peak plasma concentrations, which were obtained at delayed time post-treatments ($P < 0.05$, Table 1). However, while after the administration of 15 mg/kg dose the dose-adjusted C_{max} resulted 27% higher than that observed for the 5-mg/kg dose, this value was 28% lower compared to that achieved with the highest dose (45 mg/kg).The MRT and T½el increased according to the dosage. However, this increment reached statistical significance for the MRT value between ABZ_5 and ABZ_{45} treatments.

The plasma concentrations of $ABZSO_2$ after the i.r. administration of ABZ at different dosages to lambs are shown in Fig. 1b. The plasma pharmacokinetics parameters for $ABZSO_2$ after the i.r. administration of ABZ at different dosages to parasitized lambs are shown in Table 2. The sulphone metabolite reached C_{max} values of 0.43 ± 0.17 (ABZ_5), 1.13 ± 0.24 (ABZ_{15}) and 2.45 ± 0.63 μg/mL (ABZ_{45}) at 26.0 ± 10.5, 31.0 ± 4.52 and 61.0 ± 20.6 h post-treatment, respectively. The higher C_{max} and delayed T_{max} accounted for a significantly ($P < 0.05$) higher AUC value measured for the ABZ_{45} group (AUC = 153.6 ± 60.9 μg·h/mL), compared to those obtained in the ABZ_5 treatment (AUC = 12.9 ± 8.15 μg·h/mL). Similar to that observed for ABZSO, when the AUC values were adjusted by the dosage, enhancements of 20% ($P > 0.05$) and 33% ($P < 0.05$) for the 15 and 45 mg/kg doses, respectively, were observed. While any effect on the T½el for $ABZSO_2$ was observed among treatments, the MRT values between the ABZ_5 (30.9 ± 3.16 h) and ABZ_{45} (56.2 ± 9.77 h) were significantly different ($P < 0.05$).

Figure 2 shows the individual relationship between adult *H. contortus* counts and the pharmacokinetic parameters $AUC_{0\text{-}LOQ}$ (Fig. 2a) and C_{max} (Fig. 2b), obtained in infected animals treated with the different dosage levels. High correlations between the estimated pharmacokinetic parameters and nematode counts were observed, with Spearman's coefficients of −0.83 and −0.84 for $AUC_{0\text{-}LOQ}$ and C_{max}, respectively.

DISCUSSION

The results obtained in the experiment described here clearly show that an increase in ABZ dosage in lambs is associated with enhancement in the plasma exposure of ABZ metabolites. A different situation was described in dogs (McKellar *et al.*, 1990, 1993), where earlier pharmacokinetic studies involving FBZ showed that increasing the dose did not correlate with any increase in the amount of drug absorbed. The observed differences between ruminants and dogs in the dosage/plasma exposure relationship may heavily depend on the GI anatomy/physiology of each animal species. The water solubility of BZD compounds drastically increases at extreme pH values. As a consequence, the sheep abomasum plays a crucial role in drug absorption. When a BZD suspension is deposited in the rumen, solid particles mix and distribute through the digesta volume (Hennessy, 1993). The rumen acts as a drug reservoir by

Table 1. Plasma pharmacokinetic parameters (mean ± SD) for albendazole sulphoxide (ABZSO) obtained after the intraruminal (i.r.) administration of albendazole (ABZ) to lambs at different dosages: ABZ_5 (5 mg/kg), ABZ_{15} (15 mg/kg) and ABZ_{45} (45 mg/kg)

	ABZSO		
Pharmacokinetic parameters	ABZ_5	ABZ_{15}	ABZ_{45}
C_{max} (μg/mL)	0.90 ± 0.40[a]	4.63 ± 0.70[b]	8.00 ± 1.70[c]
T_{max} (h)	15.0 ± 6.30[a]	22.0 ± 6.20[a,b]	29.0 ± 5.90[b]
$AUC_{0\text{-}LOQ}$ (μg·h/mL)	21.0 ± 6.40[a]	158.6 ± 19.4[b]	389.7 ± 98.2[c]
$AUC_{0\text{-}\infty}$ (μg·h/mL)	22.1 ± 6.30[a]	159.6 ± 19.6[b]	426.2 ± 131.4[c]
AUMC (μg·h^2/mL)	491.1 ± 119.9[a]	4714 ± 835.8[a,b]	23659 ± 17482[b]
T½el (h)	9.31 ± 1.20[a]	10.8 ± 0.70[a]	22.9 ± 19.2[a]
MRT (h)	23.0 ± 2.50[a]	29.6 ± 4.10[b]	50.5 ± 23.9[b]
Normalized AUC*	21.0 ± 6.40[a]	52.9 ± 6.48[b]	43.3 ± 10.9[a,b]
Normalized C_{max}*	0.90 ± 0.40[a,b]	1.54 ± 0.22[a]	0.89 ± 0.19[b]

C_{max}, peak plasma concentration; T_{max}, time to the C_{max}; $AUC_{0\text{-}LOQ}$, area under the plasma concentration vs. time curve from 0 up to the quantification time; AUC_0-∞, area under the concentration vs. time curve extrapolated to infinity; AUMC, area under the first moment curve; T½el, elimination half-life; MRT, mean residence time (obtained by noncompartmental analysis of the data). *$AUC_{0\text{-}LOQ}$ and C_{max} values were dose-normalized dividing the observed value by 3 (ABZ_{15}) or 9 (ABZ_{45}). Pharmacokinetic parameters with different superscript letters are statistically different at $P < 0.05$.

Table 2. Plasma pharmacokinetic parameters (mean ± SD) for albendazole sulphone ($ABZSO_2$) obtained after the intraruminal (i.r.) administration of albendazole (ABZ) to lambs at different dosages: ABZ_5 (5 mg/kg), ABZ_{15} (15 mg/kg) and ABZ_{45} (45 mg/kg)

	$ABZSO_2$		
Pharmacokinetic parameters	ABZ_5	ABZ_{15}	ABZ_{45}
C_{max} (μg/mL)	0.43 ± 0.17[a]	1.13 ± 0.24[a, b]	2.45 ± 0.63[b]
T_{max} (h)	26.0 ± 10.5[a]	31.0 ± 4.52[a]	61.0 ± 20.6[b]
$AUC_{0\text{-}LOQ}$ (μg·h/mL)	12.9 ± 8.15[a]	46.5 ± 9.32[a, b]	153.6 ± 60.9[b]
$AUC_{0\text{-}\infty}$ (μg·h/mL)	14.0 ± 7.86[a]	48.0 ± 9.17[a, b]	154.8 ± 60.4[b]
AUMC (μg·h^2/mL)	449.8 ± 314.5[a]	1961 ± 438.1[a,b]	9137 ± 4754[b]
T½el (h)	9.59 ± 2.08[a]	12.9 ± 3.71[a]	12.1 ± 1.80[a]
MRT (h)	30.9 ± 3.16[a]	40.9 ± 5.84[a]	56.2 ± 9.77[b]
Normalized AUC*	12.9 ± 8.15[a]	15.5 ± 3.1[a]	17.1 ± 6.77[a]
Normalized C_{max}*	0.43 ± 0.17[a]	0.38 ± 0.08[a]	0.27 ± 0.07[a]

C_{max}, peak plasma concentration; T_{max}, time to the C_{max}; $AUC_{0\text{-}LOQ}$, area under the plasma concentration vs. time curve from 0 up to the quantification time; AUC_0-∞, area under the concentration vs. time curve extrapolated to infinity; AUMC, area under the first moment curve; T½el, elimination half-life; MRT, mean residence time (obtained by noncompartmental analysis of the data). *$AUC_{0\text{-}LOQ}$ and C_{max} values were dose-normalized dividing the observed value by 3 (ABZ_{15}) or 9 (ABZ_{45}). Pharmacokinetic parameters with different superscript letters are statistically different at $P < 0.05$.

slowing the digesta transit time throughout the abomasum, which results in improved systemic availability of BZD compounds as a consequence of a greater dissolution of drug particles in the acid pH of the abomasum (Lanusse & Prichard, 1993). A different situation may occur in dogs. When the BZD anthelmintic suspensions are orally administered to this monogastric species, the drug reach directly the stomach, beginning the dissolution process. However, the short gut transit time in dogs determines a shorter time for dissolution of the administered drug suspension compared to that observed in ruminants, limiting the GI absorption of the parent compound. As previously mentioned, it has been demonstrated that increasing the dosage did not significantly increase the amount of FBZ absorbed in dogs (McKellar *et al.*, 1993). A combination of poor water solubility of FBZ and short gut transit time in the dog accounts for the small differences in the AUC values of FBZ and its metabolites, observed with the increment in the dosage (McKellar *et al.*, 1990). The influence of the rumen on BZD absorption was evidenced in earlier studies, where higher and more sustained concentrations of FBZ, OFZ, ABZ and their metabolites were recovered in the bloodstream after oral/i.r. treatments compared to intra-abomasal administration of the same compounds in sheep (Prichard *et al.*, 1978; Marriner & Bogan, 1981). Data obtained in the current work clearly shown that different from the observations in monogastric species (dogs) and at least within the evaluated dose range, higher i.r. dose of ABZ in sheep accounts for a greater amount of drug absorbed at the GI level.

The ABZSO plasma concentration profiles show a dose-dependent relationship. Significantly higher plasma AUC and

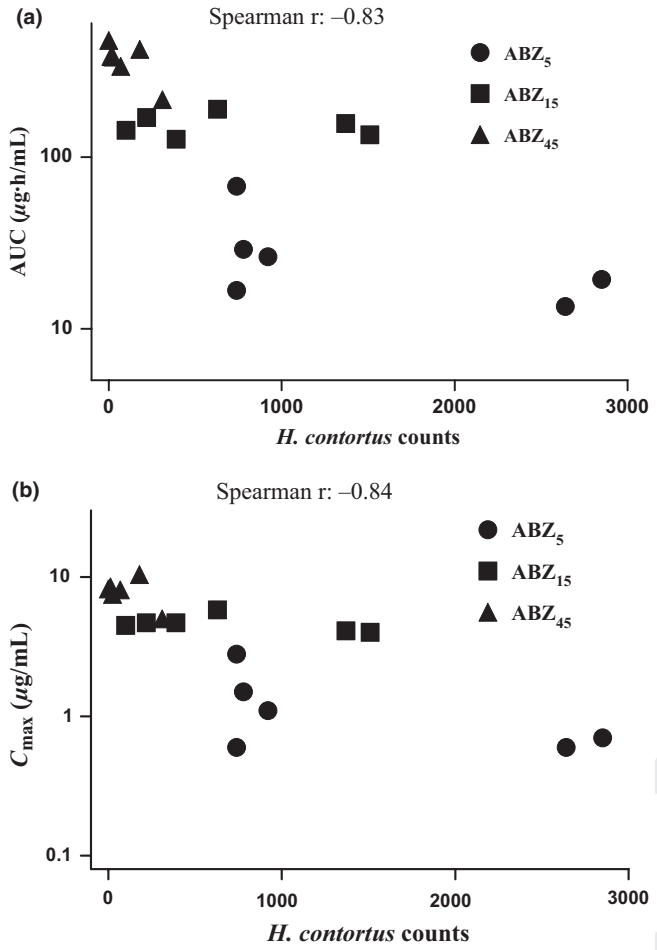

Fig. 2. Correlation between adult *Haemonchus contortus* individual counts and values of area under the concentration vs. time curve (AUC) (a), and peak plasma concentration (C_{max}) (b), in lambs treated with albendazole (ABZ) at the dose of 5 (ABZ_5), 15 (ABZ_{15}) and 45 (ABZ_{45}) mg/kg.

C_{max} values for ABZSO were observed after the administration of ABZ at both 15 and 45 mg/kg compared to the treatment at 5 mg/kg. The AUC of the active ABZSO metabolite increased from 21.0 (ABZ_5) up to 158.6 (ABZ_{15}) and 389.7 μg·h/mL (ABZ_{45}). These differences may be explained by the greater amount of drug administered at the higher doses rates. Although higher systemic exposure was obtained with the higher dosage levels, a lack of dose proportionality was observed. The dose-normalized $AUC_{0\text{-}LOQ}$ values for ABZSO after the 15-mg/kg dose was significantly greater than that observed after 5 mg/kg dose (Table 1). Additionally, a significantly longer MRT value was observed for ABZSO in both ABZ_{15} and ABZ_{45} treatments. The MRT value represents the time point at which 63.2% of the drug has been eliminated from the body (Riviere, 1999). Regardless the administered dosage, the MRT of a given drug should remain constant in the absence of a saturable kinetic process. Thus, the higher dose-normalized AUC (ABZ_{15}) and the longer MRT values (ABZ_{15} and ABZ_{45}) obtained for ABZSO reflect some type of nonproportionality in the dose–plasma concentrations relationship.

The lack of dose proportionality observed for ABZ (estimated as the ABZSO systemic exposure) may be associated with a saturation on the enzymatic pathways involved on its biotransformation. A flavin-containing monooxygenase (FMO) is mainly associated with the ABZ oxidation to form ABZSO (Galtier *et al.*, 1986; Virkel *et al.*, 2004), while the cytochrome P-450 system (CYP) is involved in the second, slower and irreversible oxidative step by which ABZSO is converted into $ABZSO_2$ (Souhaili-El-Amri *et al.*, 1988). It has been demonstrated that the FMO-mediated sulphoxidation accounted for up to 60% of the ABZSO production from ABZ, while the CYP contributed with 40% in sheep liver microsomes (Virkel *et al.*, 2004). Thus, a decreased metabolic rate for the conversion of ABZSO into $ABZSO_2$ may have accounted for the marked changes observed on the ABZSO disposition kinetics after the administration of three times (ABZ_{15}) the therapeutic dose. This finding agrees with previously generated data (Lanusse *et al.*, 1992), where significant higher AUC and longer MRT values for ABZSO were obtained when methimazole, a FLO-metabolic inhibitor, was co-administered with netobimin (a pro-BZD biotransformed to ABZ) in sheep.

Furthermore, the administered ABZ dosage level may have caused some kinetic interference at the GI tract. The existence of synergistic effects between metabolic enzymes and efflux transporters at the intestinal level has been suggested (Suzuki & Sugiyama, 2000). The CYP system and the permeability glycoprotein (Pgp) may act synergistically in reducing the bioavailability of their substrates after oral administration. In the case of ABZ, after being taken up by the enterocytes, some of the parent drug suffers a CYP-mediated (mainly CYP3A or CYP1A) first intestinal oxidative step. The ABZSO formed may be secreted from the cell into the intestinal lumen via drug transporter proteins. It is known that ABZSO is actively secreted into the intestinal lumen (Redondo *et al.*, 1999), likely due to a combination of passive diffusion and active transport. Pgp, multidrug resistance protein 2 (MRP2) and the breast cancer resistance protein have been proposed as the main candidate proteins involved on ABZSO intestinal efflux transport (Merino *et al.*, 2003). A highly efficient transport of ABZSO by murine BCRP1 has been described (Merino *et al.*, 2005). In contrast, ABZ parent drug does not seem to interact with Pgp (Merino *et al.*, 2002, 2005; Dupuy *et al.*, 2010), MRP2 or BCRP1 (Merino *et al.*, 2005). Thus, the large amounts of ABZSO available at the small intestine level following treatment at three and ninefold the therapeutic dose may have saturated the efflux pumping capacity of the transporters, accounting for the observed enhanced ABZSO systemic availability.

No differences ($P > 0.05$) in both dose-normalized AUC and C_{max} were observed between ABZ_5 and ABZ_{45} treatments. Dissolution is a crucial step as drug particles must dissolve in the enteric fluids to allow absorption through the GI mucosa. The undissolved drug particles passing down the GI tract in the luminal content are excreted in faeces without exerting its action. It is likely that a proportionally low dissolution rate may be associated with the administered highest ABZ dose. Additionally, as previously reported for oxfendazole in goats (Sangster *et al.*, 1991), a delayed T_{max} and longer MRT values observed for

ABZSO in the experimental animals treated at the highest dosage 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.

The overall kinetic disposition of the inactive sulphone metabolite did not change after treatment at threefold the therapeutic dosage. Dose-normalized AUC and C_{max} values did not differ ($P > 0.05$) between the ABZ_5 and ABZ_{15} treatments. However, the $ABZSO_2$ plasma disposition kinetics was altered in the ABZ_{45} treatment. Significantly ($P < 0.05$) higher AUC, C_{max} and MRT values were observed after administration at the highest dosage level. Additionally, the delayed appearance of $ABZSO_2$ in the bloodstream resulted in significantly ($P < 0.05$) longer T_{max} in the ABZ_{45} (61.0 ± 20.6 h) compared to that observed in the ABZ_5 experimental group (26.0 ± 10.5 h). The delayed $ABZSO_2$ T_{max} may be a consequence of the reduction in the rate of conversion of ABZ to ABZSO and then to $ABZSO_2$.

As expected, there was a highly negative correlation between the ABZSO AUC and C_{max} values, and the number of adult *H. contortus* recovered from treated lambs. The enhanced systemic exposure achieved after ABZ treatments at the highest dosages correlated with significant increment in drug efficacy against a resistant *H. contortus* strain. In fact, the efficacies against resistant *H. contortus* were 16% (ABZ_5), 59% (ABZ_{15}) and 94% (ABZ_{45}) (V. Barrère et al., submitted). BZD resistance has been correlated to genetic changes associated with the ß-tubulin gene (Lubega & Prichard, 1990). These changes, mainly focused on positions 200 and 167 of the ß-tubulin gene, determine a reduced binding affinity, which explains the development of anthelmintic resistance to BZD compounds (Beech *et al.*, 1994; Kwa *et al.*, 1994). A loss of receptor affinity increases the concentration of drug needed for a given degree of response. The observed results indicate that higher active drug/metabolite concentrations associated with the highest ABZ dosages, resulted in significant increased drug efficacy. As BZD metabolites are reversibly exchanged between the bloodstream and the GI tract (Lanusse *et al.*, 1993), the enhanced drug concentrations associated with the increasing administered doses may account for GI nematodes being exposed to toxic drug concentrations for extended period of time. This finding helps to explain the reversion of the drug resistance phenomenon observed in the current trial after administration of ABZ at high dose level. In fact, according to the WAAVP guidelines, the efficacy of the different ABZ treatments against resistant-*H. contortus* changed from 'insufficiently active' (<80%) in lambs treated with 5 or 15 mg/kg, to 'effective' (90–98%) after treatment with the highest dose.

The work reported here contributes to the understanding of the pharmacokinetic impact of using high BZD anthelmintic doses to control resistant helminth parasites. As it was shown here, the BZD resistance mechanism based on a reduced drug affinity at its receptor binding site may be overcome by increasing active drug concentrations at the biophase. In fact, under our experimental conditions, an ABZ dose as high as 9 fold the therapeutic dosage was necessary to reach an acceptable efficacy level against resistant *H. contortus*. The inconvenience of recommending high dosages may be associated with the selection of highly resistant nematodes, in addition to the impact on drug residues, withdrawal times, etc. A pharmacokinetic contribution to the issue is the main outcome of the work described here.

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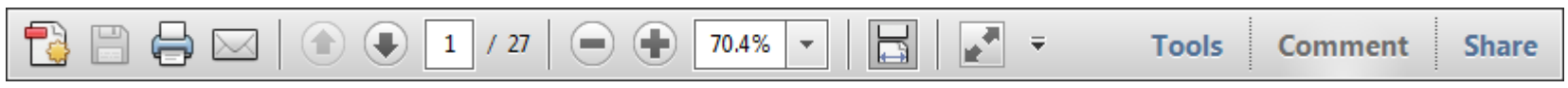

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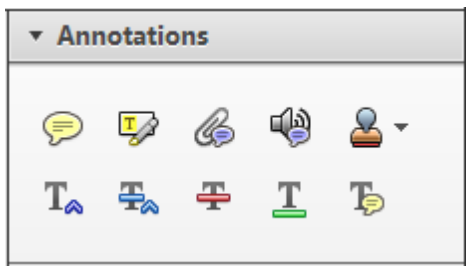

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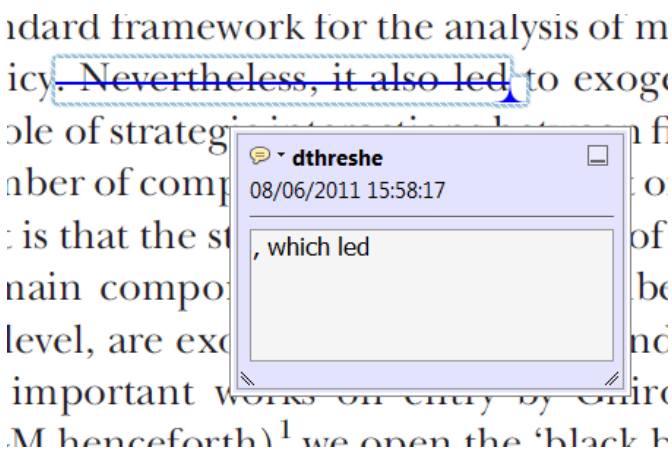

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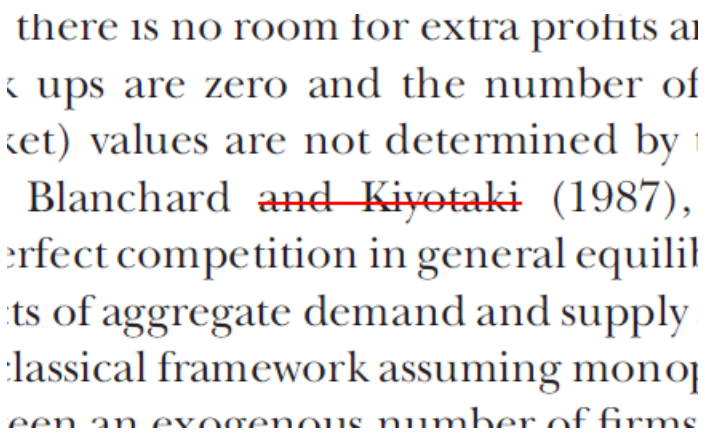

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- Click on the Add note to text icon in the Annotations section.
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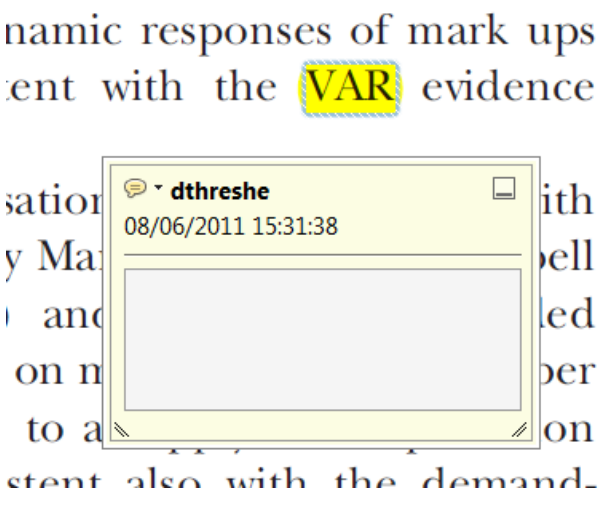

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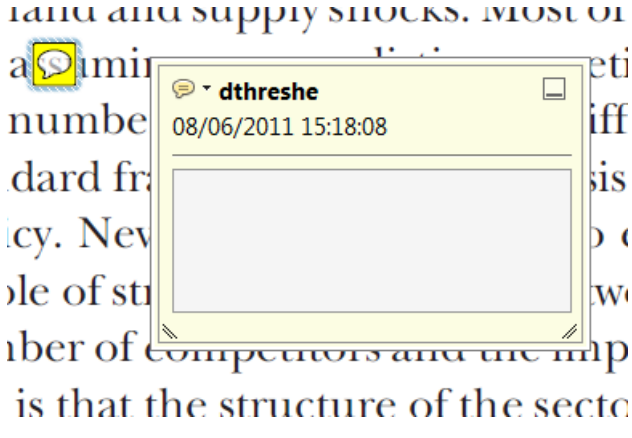

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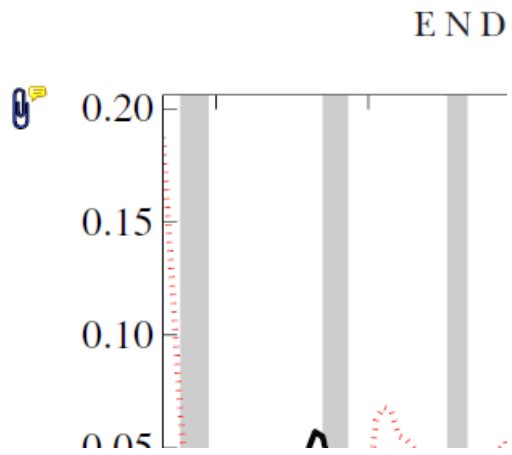

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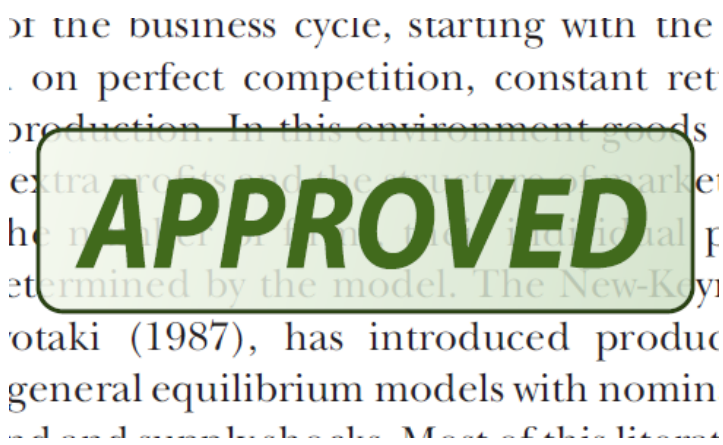

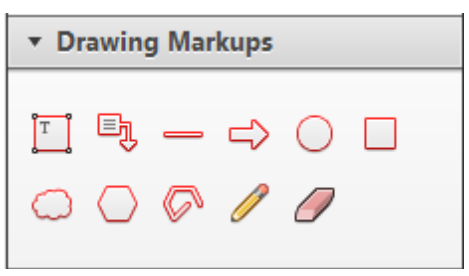

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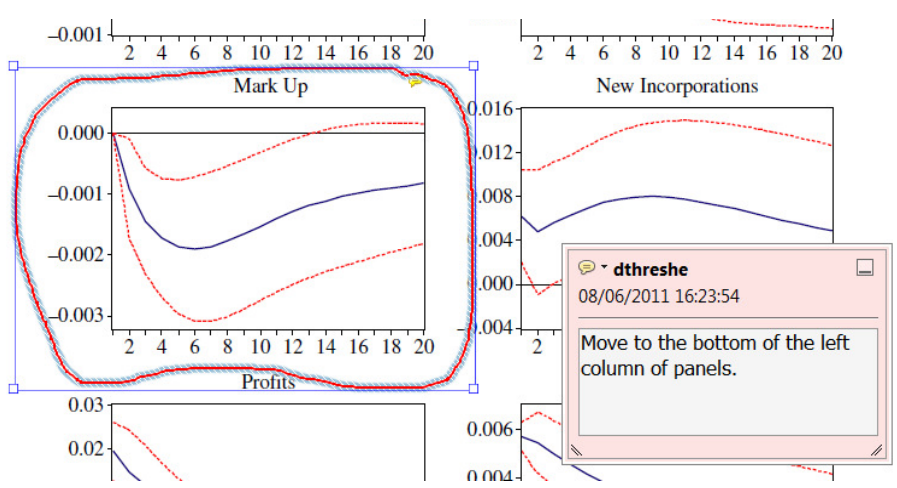

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