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Combined use of ivermectin and triclabendazole in sheep: In vitro and in vivo characterisation of their pharmacological interaction

Adrian Lifschitz [a,b,*], Guillermo Virkel [a,b], Mariana Ballent [a,b], Juan Sallovitz [a], Carlos Lanusse [a,b]

[a] *Laboratorio de Farmacología, Facultad de Ciencias Veterinarias, Universidad Nacional del Centro de la Provincia de Buenos Aires (UNCPBA), Campus Universitario, 7000 Tandil, Argentina*
[b] *Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina*

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Abstract

This study evaluated the pharmacokinetic properties of ivermectin (IVM) and triclabendazole (TCBZ) given either separately or co-administered to sheep. Corriedale sheep received IVM alone, TCBZ alone or a combination of IVM and TCBZ intravenously. Ivermectin elimination was delayed and its plasma availability was 3-fold higher when co-administered with TCBZ. Similarly, plasma concentrations of TCBZ and its metabolites were influenced by the co-administration of IVM. Higher peak plasma concentrations of TCBZ metabolites were detected after the co-administration of TCBZ and IVM compared to those obtained following TCBZ treatment in isolation. Complementary in vitro assays were carried out to assess the influence of TCBZ on the P-glycoprotein-mediated intestinal transport of IVM, using the everted gut sac technique. Enhanced accumulation of IVM in the intestinal wall occurred after co-incubation with TCBZ.

Keywords: Ivermectin; Triclabendazole; Pharmacokinetic interaction; Sheep; Everted gut sac technique

Introduction

The pharmaco-toxicological effects of xenobiotic compounds are highly dependent on their pharmacokinetic (PK) properties such as absorption characteristics, tissue distribution and rate of metabolism and elimination. The concomitant administration of multiple drugs is often used in veterinary therapy and may affect their kinetics and pharmacological activity. Drug interactions may result in alterations in their metabolism or excretion or their displacement from binding proteins (Lin, 2007). In particular, there is increasing evidence to suggest that interference between drugs and ATP binding cassette (ABC) and other transport proteins is a key mechanism underpinning clinically important drug interactions (Marchetti et al., 2007).

Ivermectin (IVM) is a semi-synthetic broad-spectrum avermectin active against endo- and ecto-parasites of clinical significance in both veterinary and human medicine. Ivermectin is available in injectable, oral and 'pour-on' formulations for use in animals (McKellar and Benchaoui, 1996). As IVM is not effective against trematodes (Campbell and Benz, 1984), it is frequently used in combination with trematodicidal compounds to extend its antiparasitic spectrum of activity and commercial formulations that include IVM combined with clorsulon, nitroxynil or triclabendazole are available. PK and/or pharmacodynamic (PD) interactions between these components may thus occur and the potential therapeutic consequences of these interactions require study.

* Corresponding author. Address: Laboratorio de Farmacología, Facultad de Ciencias Veterinarias, Universidad Nacional del Centro de la Provincia de Buenos Aires (UNCPBA), Campus Universitario, 7000 Tandil, Argentina. Tel./fax: +54 2293 439850.
E-mail address: adrianl@vet.unicen.edu.ar (A. Lifschitz).

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An interaction between IVM and the ABC transporter P-glycoprotein (P-gp) has been demonstrated in vitro (Didier and Loor, 1996; Ballent et al., 2006; Lespine et al., 2007). Recent work at our laboratory found that the co-administration of IVM and either loperamide or itraconazole, as P-gp modulators, resulted in enhanced availability of the anthelmintic in both the blood and tissues of rats (Lifschitz et al., 2004), cattle (Lifschitz et al., 2007) and sheep (Ballent et al., 2007). Triclabendazole (TCBZ) is a halogenated benzimidazole (BZD) anthelmintic used worldwide to control immature and adult stages of the liver fluke *Fasciola hepatica* (Boray et al., 1983). It is formulated as an oral suspension for sheep and cattle and it has recently been shown that TCBZ and its sulfoxide metabolite (TCBZSO) inhibit P-gp transport in cultured cells transfected with the ABC transporter (Dupuy et al., 2006).

In view of the growing, widespread use of combined anthelmintic therapy, the current study set out to evaluate the pharmacokinetics of IVM and TCBZ given either separately or co-administered to sheep. To further elucidate the kinetic interactions of these compounds, complementary in vitro assays were established to assess the influence of TCBZ and its metabolites on the P-gp-mediated intestinal secretion of IVM.

Materials and methods

Experimental animals and procedures

Animal procedures and management protocols were carried out in accordance with 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[1].

In vivo pharmacokinetic trial

The study was carried out using 15 clinically normal, parasite-free Corriedale sheep of between 25 and 30 kg bodyweight, grazing lucerne-red clover pasture with free access to water. The health of the animals was monitored prior to and throughout the experimental period.

The sheep were randomly allocated to three groups of five. Group A animals received IVM by intravenous (IV) injection at 50 μg/kg (Ivosint, Biogénesis). The original IVM formulation was diluted 1:4 in propylene glycol to accommodate the low dose volume used. Group B sheep were treated with TCBZ by IV injection at 5 mg/kg with the TCBZ diluted with propylene glycol:DMSO (90:10). Group C animals received IVM at 50 μg/kg and TCBZ at 5 mg/kg, by two separate IV injections. Jugular blood was collected into heparinised Vacutainers 10, 20, 30 and 60 min, 2, 4, 8, 12, 24, 36 and 48 h and 3, 4, 5 and 7 days post-treatment. Blood samples were centrifuged at 2000*g* for 20 min. The plasma obtained was stored at −20 °C until analysed.

In vitro gut transport assays

The everted gut sac assay system was established as previously described (Barthe et al., 1998). In brief, food was withdrawn overnight from male rats weighing between 250 and 300 g. Under anaesthesia, the intestine was rapidly removed from the animals and washed in a buffer solution consisting of: 1 mM $NaH_2PO_4 \cdot H_2O$; 2.5 mM $CaCl_2 \cdot H_2O$; 4.7 mM KCl; 1.1 mM $MgCl_2 \cdot 6H_2O$; 0.004 mM EDTA; 11 mM glucose; 119 mM NaCl, 25 mM Na_2CO_3; and 0.11 mM ascorbic acid. The intestine was then immediately placed in warm (37 °C), oxygenated (O_2/CO_2, 95%/5%), 199 medium (Sigma–Aldrich) and gently everted over a 2.5 mm diameter glass rod. One end of the intestine was clamped and sutured with silk braid before it was filled with medium at 37 °C using a 1 mL plastic syringe. The intestinal segment was then sealed with a second silk braid suture. Such sacs, 5 cm in length, were placed in individual incubation chambers containing 6 mL of pre-gassed oxygenated media at 37 °C.

Incubation assays

To study tissue uptake of IVM in the presence or absence of TCBZ and TCBZSO (Microsules), 0.5 μM IVM, 8 μM TCBZ and 8 μM TCBZSO were added to the chambers. Sacs were incubated in a water bath for 30, 60 and 90 min. The solution was maintained at 37 °C with O_2/CO_2 (95%/5%) throughout the experiment. At the defined time points, sacs were removed, washed in buffer solution and blotted dry. The sacs were opened and the serosal fluid collected into small tubes. Each sac was weighed before and after fluid collection to calculate the volume inside the sac. All samples were stored at −20 °C until analysed for IVM by high performance liquid chromatography (HPLC).

Gut sac viability

To verify the integrity of the gut sacs, glucose concentrations were measured both in the incubation medium and in the sac contents using a commercial assay (Wiener). Since glucose is actively transported by the small intestine, intact healthy and metabolically active sacs will concentrate glucose in the serosal medium. The sacs were incubated in TC 199 medium in the chambers in the presence and absence of the drugs under test and while maintaining the same conditions as in the experimental assay. The gut sacs were incubated for between 10 and 90 min before samples of the incubation medium and the content of the sacs were collected and stored for subsequent glucose determination. Glucose determination was carried out as follows: 20 μL of sample was incubated at 37 °C for 10 min with 50% distilled water, 5% 25 mM/L 4-aminofenazona solution in Tris buffer (0.92 M/L); 5% 55 mM/L phenol solution and 0.3% of 1000 U/mL glucose oxidase in 120 U/mL peroxidase. The absorbance was then measured using a spectrophotometer at 505 nm.

Analytical procedures

Ivermectin analysis

Ivermectin was extracted from plasma as previously described (Alvinerie et al., 1993 as adapted by Lifschitz et al., 2000). Aliquots of plasma (1 mL) and 0.25 g of intestinal wall were fortified with 10 μL of abamectin (1 μg/mL) (used as an internal standard) and acetonitrile (1 mL and 0.25 mL, respectively). Deionised water (0.250 mL and 0.062 mL, respectively) was added to each sample. The preparation was mixed using a MultiTube vortexer (VWR Scientific Products) for 15 min, sonicated in an ultrasonic bath for 10 min (Transsonic 570/H, Laboratory Line Instruments) and the mixture was then centrifuged at 2000 *g* for 10 min. The supernatant was manually transferred into a tube and the procedure was repeated once for the intestinal tissue samples. The supernatant was applied to a conditioned Supelclean LC 18 cartridge (Supelco).

After washing with 1 mL of deionised water followed by 1 mL of water–methanol (4:1 v/v), the cartridges were dried for 5 min and the sample eluted with 1.5 mL methanol, which was collected. After solid phase extraction, the elute was evaporated under a gentle stream of dry nitrogen at 60 °C in a water bath. The sample was then subjected to derivatisation as described by De Montigny et al. (1990). After completion of the reaction, a 100 μL aliquot of each sample was injected directly into the chromatographic system.

High performance liquid chromatography (HPLC) and validation procedures

Ivermectin concentrations in plasma and intestinal tissue were measured by HPLC (Shimadzu 10A HPLC system) as previously described (Lifschitz et al., 2000). The chromatographic conditions included a mobile

[1] See: http://www.vet.unicen.edu.ar.

phase of acetic acid (0.2% in water)–methanol–acetonitrile (2.4:40:57.6 v/v/v) pumped at a flow rate of 1.5 mL/min through a reverse phase C_{18} column (Thermoquest, Hypersil) of dimensions 5 μm by 4.6 mm by 250 mm. Detection of the IVM was effected using a fluorescence detector (Spectrofluorometric detector RF-10, Shimadzu) set at an excitation wavelength of 365 nm and an emission wavelength of 475 nm. The IVM/ABM peak area ratio was used to estimate the IVM concentration in spiked (for validation of the analytical method) and experimental samples. There was no interference by endogenous compounds in the chromatographic analysis. The analytical procedures, including the chemical extraction and HPLC analysis of IVM in both the plasma and intestinal wall were validated.

The statistical program Instat 3.0 (Graph Pad Software Inc.) was used for linear regression analyses and linearity tests. Calibration curves were prepared in a range between 0.2 and 100 ng/mL for plasma and between 0.010 and 2.5 nM/g (8.75–2187 ng/g) for intestinal wall. Linearity was established to express the concentration-detector response relationship, as determined by injection of plasma and tissue IVM spiked standards at different concentrations (three replicates). Calibration curves were established using least squares linear regression analysis and the correlation coefficient (r) and coefficient of variation (CV) were calculated.

Drug recovery was estimated by comparing the peak area from spiked plasma and tissue standards at different concentrations, with the peak areas resulting from direct injections of IVM standards in methanol. The limit of quantification was established as the lowest concentration measured with a recovery >70% and a CV of <20%. The CV in the procedures for plasma and intestinal wall, obtained after HPLC analysis of IVM spiked standards on different working days, was between 3.5% and 5.9%. The linear regression lines for tissues analysed indicated r to be $\geqslant 0.998$. The mean recoveries of IVM were up to 70% (plasma and intestinal wall). The limit of quantification was established at 0.2 ng/mL for plasma and 0.01 μM for intestinal wall.

Analysis of triclabendazole and its metabolites

Extraction of TCBZ and its metabolites from plasma was carried out as previously described (Virkel et al., 2006). Aliquots of plasma (0.5 mL) were supplemented with oxibendazole (OBZ) (used as an internal standard, 99.2% pure with 1 μg/40 μL methanol) and then mixed with 2 mL acetonitrile. The samples were shaken for 20 min in a multivortex and then centrifuged at 2500*g* for 15 min. Supernatants were evaporated using an Automatic Environmental Speed Vac System (Savant). The dry residue was re-suspended in a 300 μL mobile phase and 50 μL of this solution were injected into the HPLC system.

Analysis of TCBZ and its metabolites by HPLC was carried out as described by Virkel et al. (2006). Fifty microlitres of each extracted sample were injected through an autosampler (Shimadzu SIL 10 A Automatic Sample Injector) into a Shimadzu 10 A HPLC system fitted with a Kromasil C_{18} (5 μm, 250 mm by 4.60 mm) reverse-phase column (Eka Chemicals) and UV detector (Shimadzu, SPD-10A UV detector) reading at 292 nm. The mobile phase was an acetonitrile–ammonium acetate solution (0.025 M, pH 6.6) with elution gradient (0–5 min: 52/48; 6–12 min: 64/36; 13–16 min: 52/48). Chromatographic peak areas for each molecule were measured using the integrator software (Class LC 10, Shimadzu Corporation) of the HPLC system.

Validation of the analytical procedures for the extraction and quantification of TCBZ and its metabolites was performed before analysis of the experimental samples from the incubation assays. Between 0.05 and 10.0 μg/mL of each analyte were added to aliquots of plasma from untreated sheep. Fortified samples were extracted and analysed by HPLC (triplicate determinations) to obtain calibration curves and recovery percentages. Calibration curves were analysed using least squares linear regression analysis (Instat 3.00, Graph Pad Software Inc.) of HPLC peak area ratios of analytes/IS and nominal concentrations of spiked samples. Correlation coefficients for the different analytes ranged between 0.995 and 0.999. A lack-of-fit test was also carried out to confirm the linearity of the regression line of each analyte. The concentrations in the experimental samples were determined following interpolation using the standard curves.

Absolute recoveries were established in a concentration range of between 0.05 and 2 μg/mL. The absolute recoveries of drug and metabolites from plasma were: 82–87% for TCBZ sulfone, 86–91% for TCBZSO, and 76–87% for TCBZ, respectively. The inter-assay precision of the extraction and chromatography procedures was estimated by processing replicate aliquots ($n = 4$) of pooled sheep plasma samples containing known concentrations of each molecule (0.5, 1 and 2 μg/mL) on different days. Coefficients of variation of <20% were obtained when the inter-day precision of the chromatographic method was evaluated for the different analytes.

Pharmacokinetic and statistical analyses

The concentration versus time curves for TCBZ and its metabolites and for IVM in plasma for each individual animal after the different treatments were fitted using PK Solution 2.0 (Summit Research Services). The data points generated for TCBZ or IVM in plasma after administration were best-fitted to a two-compartment model (Notari, 1987) according to the equation:

$$C_p = Ae^{-\alpha t} - Be^{-\beta t},$$

where A and B are the primary and secondary disposition intercepts, α and β are the primary and secondary disposition rate constants, and C_p is the plasma concentration of TCBZ or IVM at time t. The distribution and elimination half-lives were calculated as *ln2* divided by the rate constants. The estimated plasma concentration of TCBZ and IVM parent drug at zero time (C_p^0) after administration was the sum of the coefficients A and B. Total body clearance (Cl_B) was calculated by:

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

where AUC represents the area under the concentration time-curve. The volume of distribution (Vd_{area}) was estimated by the following equation:

$$Vd_{area} = \text{Dose}/(\text{AUC})(\beta).$$

The further equation (Notari, 1987) was used to describe the bi-exponential concentration-time curves for TCBZSO and TCBZ sulfone after the TCBZ administration:

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

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 the first order metabolite formation rate constant (k_{for}) (h^{-1}). The elimination half-life ($T_{½el}$) and metabolite formation half-life ($T_{½for}$) were calculated as $ln2/\beta$ and $ln2/k$, respectively. The peak concentration (C_{max}) and time to peak concentration (T_{max}) were displayed 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). The mean residence time (MRT) was determined as AUMC/AUC (Perrier and Mayersohn, 1982) where AUMC is the area under the curve of the product of time and the plasma drug concentration against time from zero to infinity (Gibaldi and Perrier, 1982) and the AUC is as defined above.

Drug concentrations and all PK parameters are reported as mean ± standard deviations (SD). A normality test was performed to assess if the data sampled followed a Gaussian distribution. This assumption was tested using the Kolmogorov and Smirnov method. The mean PK for different experimental groups was compared using The Student's t test. The assumption that the data obtained after both treatments had the same variance was assessed. A non-parametric Mann–Whitney test was used where significant differences among SD were observed. A value of $P < 0.05$ was considered statistically significant.

Results

Ivermectin was detected in plasma between 10 min and 7 days after IV administration and its plasma concentration

profile was affected by the co-administration of TCBZ. Contemporaneous administration of the flukicide resulted in higher plasma concentrations of IVM when compared to the control group, particularly between 2 and 7 days post-treatment. The IVM plasma concentration profiles obtained with and without co-administration of TCBZ are compared in Fig. 1. The IVM AUC values were 3.13-fold higher in the presence of TCBZ.

Despite the increased IVM plasma concentrations found following co-administration of TCBZ, no statistically significant difference was found in IVM systemic availability in either group due to the wide variability observed. The $T_{½el}$ and MRT for IVM were between 63% and 81% longer, respectively, when co-administered with TCBZ ($P < 0.05$). The PK parameters obtained for IVM in plasma after both treatments are summarised in Table 1.

Triclabendazole was detected in plasma between 10 min and 12 h post-administration. After administration, the sulfoxide (TCBZSO) and sulfone ($TCBZSO_2$) metabolites of TCBZ were detected in plasma up to 96 and 144 h post-treatment, respectively. The plasma concentration profile of TCBZ and its metabolites was also altered by co-administration of IVM. Triclabendazole plasma availability was lower after co-administration of IVM. The comparative plasma concentration profiles of TCBZ following administration on its own or together with IVM are shown in Fig. 2.

The co-administration of TCBZ with IVM resulted in higher TCBZSO plasma concentration profiles during the first 12 h post-treatment. Higher C_{max} for TCBZSO and $TCBZSO_2$ were obtained after co-administration of TCBZ with IVM (23.2 and 10.4 μg/mL, respectively) compared to those obtained after treatment with TCBZ alone (12.6 and 7.00 μg/mL, respectively). The MRT for TCBZSO and $TCBZSO_2$ were between 35% and 61% longer following treatment with TCBZ alone compared to when it was given in combination with IVM. The comparative concentration profiles of TCBZSO and $TCBZSO_2$, obtained after TCBZ administration either alone or in combination with IVM are illustrated in Figs. 3 and 4. Tables 2 and 3 summarise the PK parameters calculated for TCBZSO and $TCBZSO_2$ after administration of TCBZ alone or in combination with IVM.

The ability of the everted intestinal sacs to concentrate glucose by active transport across the intestine wall was used as an indicator of tissue viability. The ratio of the glucose concentration in the sac contents and incubation med-

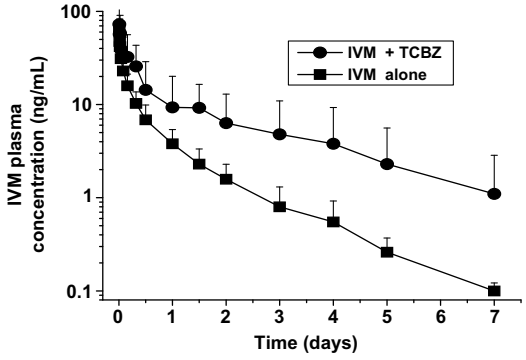

Fig. 1. Mean (±SD) ivermectin (IVM) concentration in plasma following IV administration either alone or in combination with triclabendazole (TCBZ) to sheep.

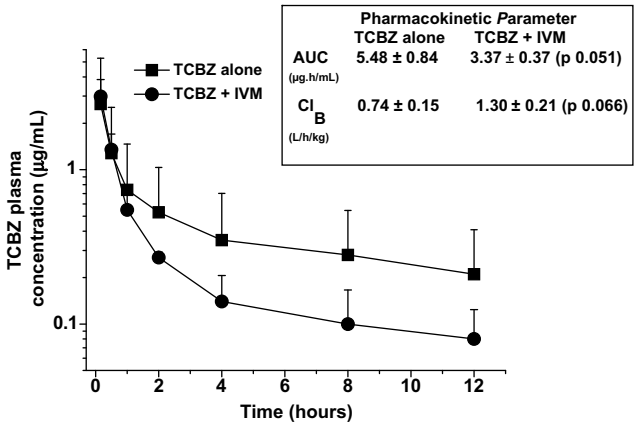

Fig. 2. Mean (±SD) triclabendazole (TCBZ) concentration in plasma following IV administration either alone or in combination with ivermectin (IVM) to sheep. The insert shows the mean value of the area under the concentration-time curve (AUC) and the total body clearance (Cl_B) obtained after both treatments.

Table 1
Means (±SDs) of the PK parameters for ivermectin (IVM) in plasma obtained after IV administration either alone or in combination with triclabendazole (TCBZ) to sheep

Kinetic parameter	IVM	IVM and TCBZ	% variation alone treatment	*P* value
$C_{initial}$	57.2 ± 16.3	59.6 ± 25.7	+4.2	0.86
AUC_{total} (ng d/mL)	14.4 ± 5.83	48.5 ± 46.6	+236	0.09
$AUMC_{total}$ (ng d/mL)	14.4 ± 7.20	113 ± 154	+684	0.09
$T_{½el}$ (d)	0.94 ± 0.04	1.53 ± 0.52	+63	0.03
MRT (d)	0.97 ± 0.10	1.76 ± 0.74	+81	0.03
Vd_{area} (L/kg)	5.33 ± 1.90	4.59 ± 3.87	−14	0.69
Cl_B (L/kg d)	3.90 ± 1.32	2.06 ± 2.00	−47	0.09

$C_{initial}$, concentration at time 0; AUC_{total}, area under the concentration-time curve extrapolated to infinity; $AUMC_{total}$, area under the first-moment concentration-time curve extrapolated to infinity; $T_{½el}$, elimination half-life; MRT, mean residence time; Vd_{area}, volume of distribution; Cl_B, total body clearance.
Mean kinetic parameters are considered significantly different from those obtained for the IVM treatment alone when $P < 0.05$.

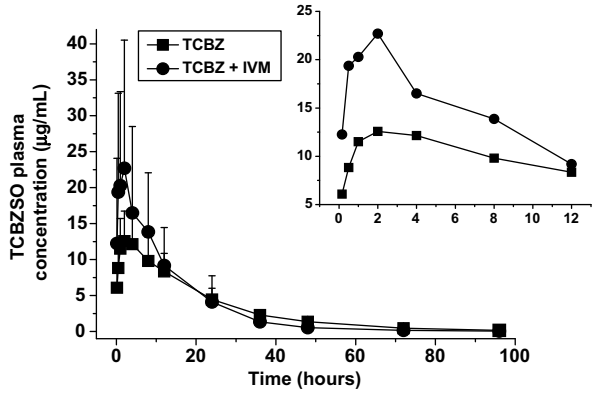

Fig. 3. Mean (±SD) triclabendazole sulfoxide (TCBZSO) concentration in plasma obtained following IV administration of triclabendazole (TCBZ) either alone or in combination with ivermectin (IVM) to sheep. The insert shows the plasma concentration profiles of TCBZSO obtained during the first 12 h post-administration.

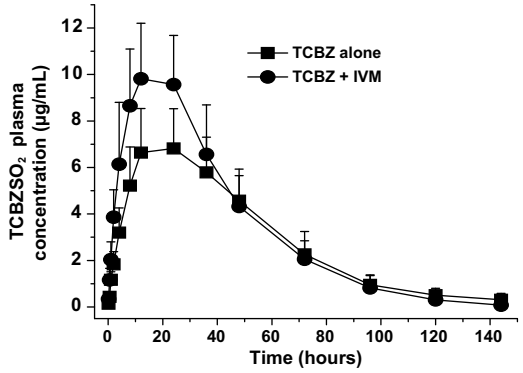

Fig. 4. Mean (±SD) triclabendazole sulfone ($TCBZSO_2$) concentration in plasma obtained following IV administration of triclabendazole (TCBZ) either alone or in combination with ivermectin (IVM) to sheep.

Table 2
Means (±SDs) of the PK parameters for triclabendazole sulfoxide (TCBZSO) in plasma obtained after the IV administration of either triclabendazole (TCBZ) alone or in combination with ivermectin (IVM) to sheep

Kinetic parameter	Treatment	
	TCBZ	TCBZ + IVM
$T_{½for}$ (h)	0.30 ± 0.15	0.56 ± 0.37
T_{max} (h)	2.80 ± 1.05	1.50 ± 0.68
C_{max} (µg/mL)	12.6 ± 4.62	23.2 ± 7.78*
$AUC_{0–12\ h}$ (µg h/mL)	125 ± 39.6	187 ± 42.4*
AUC_{total} (µg h/mL)	297 ± 74.3	319 ± 70.2
$T_{½el}$ (h)	16.7 ± 4.71	10.8 ± 1.03*
MRT (h)	21.3 ± 3.96	13.2 ± 2.13*
Ratio $AUC_{0–12\ h}$ TCBZSO/TCBZ	24.7 ± 8.58	59.7 ± 23.5*

$T_{½for}$, metabolite formation half-life; T_{max}, time to peak plasma concentration; C_{max}, peak plasma concentration; $AUC_{0–12\ h}$, area under the concentration-time curve between treatment and 12 h post administration; AUC_{total}, area under the concentration-time curve extrapolated to infinity; $T_{½el}$, elimination half-life; MRT, mean residence time; ratio $AUC_{0–12\ h}$ TCBZSO/TCBZ, ratio between $AUC_{0–12\ h}$ obtained for TCBZSO and TCBZ.

* Mean kinetic parameters are significantly different from those obtained for the TCBZ alone treatment where $P < 0.05$.

Table 3
Means (±SDs) of the PK parameters for triclabendazole sulfone ($TCBZSO_2$) in plasma obtained after the IV administration of either triclabendazole (TCBZ) alone or in combination with ivermectin (IVM) to sheep

Kinetic parameter	Treatment	
	TCBZ	TCBZ + IVM
$T_{½for}$ (h)	5.69 ± 2.07	6.87 ± 1.72
T_{max} (h)	21.6 ± 5.26	16.0 ± 7.35
C_{max} (µg/mL)	7.00 ± 1.89	10.4 ± 2.11*
$AUC_{0–24\ h}$ (µg h/mL)	127 ± 35.2	196 ± 47.3*
AUC_{total} (µg h/mL)	438 ± 85.8	489 ± 146
$T_{½el}$ (h)	29.6 ± 11.4	16.3 ± 2.31*
MRT (h)	52.3 ± 15.2	33.9 ± 4.88*
Ratio $AUC_{0–24\ h}$ $TCBZSO_2$/TCBZSO	0.64 ± 0.06	0.75 ± 0.11

$T_{½for}$, metabolite formation half-life; T_{max}, time to peak plasma concentration; C_{max}, peak plasma concentration; $AUC_{0–12\ h}$, area under the concentration-time curve between treatment and 24 h post administration; AUC_{total} ,area under the concentration-time curve extrapolated to infinity; $T_{½el}$, elimination half-life; MRT, mean residence time; ratio $AUC_{0–24\ h}$ $TCBZSO_2$/TCBZSO, ratio between $AUC_{0–24\ h}$ obtained for $TCBZSO_2$ and TCBZSO.

* Mean kinetic parameters are significantly different from those obtained for the TCBZ treatment alone where $P < 0.05$.

ium increased gradually with time up to 90 min of incubation (Fig. 5), indicating adequate tissue viability.

Ivermectin accumulated in the intestinal tissue of the sacs during the 90 min incubation. Higher IVM concentrations in the intestinal wall were found following incubation with TCBZ compared to incubation with IVM alone. Ivermectin concentrations in the wall tended to be higher after co-incubation with TCBZSO but these differences were not statistically significant. After 90 min of incubation the IVM concentration in the wall was 48% higher in the presence of TCBZ.

The ivermectin concentration profiles measured in the intestinal wall after its incubation alone or in the presence of either TCBZ or TCBZSO, are illustrated in Fig. 6. The accumulation rate of ivermectin in the wall was higher after its incubation with TCBZ (3.0 nM/cm^2/min) than with TCBZSO (2.45 nM/cm^2/min) or with IVM alone (2.15 nM/cm^2/min).

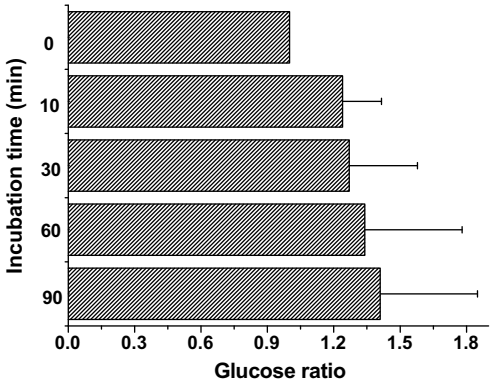

Fig. 5. The mean ratio (±SD) between the glucose concentration (g/L) in everted gut sac contents and in the incubation medium, as an indicator of intestinal tissue viability.

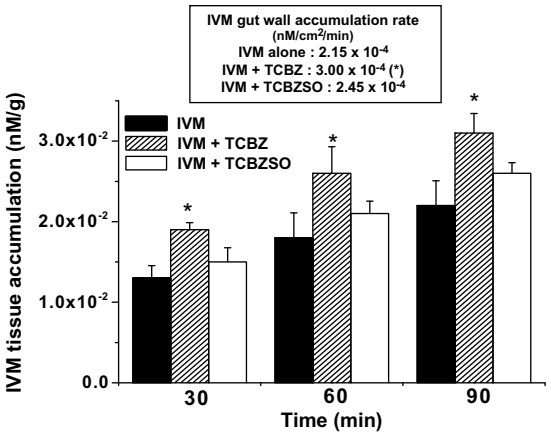

Fig. 6. Mean ivermectin (IVM) accumulation (±SD) in the wall of the everted gut sac after incubation alone, with triclabendazole (TCBZ) or with triclabendazole sulfoxide (TCBZSO), respectively. * Values are considered statistically different from those obtained following incubation with IVM alone where $P < 0.05$.

Discussion

Metabolism and transport-based drug interactions are increasingly being documented (Benet et al., 2003; Lin, 2003). Although less common than cytochrome P-450-mediated interactions, clinically significant drug interactions associated with the ABC transporter protein P-gp have been reported (Lin, 2007). The current study demonstrates that the plasma concentration profile of both antiparasitic drugs was influenced by their co-administration and the described in vivo and in vitro kinetic assessments enhance our understanding of the pharmacological interactions between these compounds.

Our findings indicated that when co-administered with TCBZ, IVM plasma concentrations were increased and had a longer MRT when compared to these parameters when IVM was administered alone. Given that IVM is excreted through the biliary and intestinal tracts as the unchanged parent drug (Chiu et al., 1990; Lifschitz et al., 2000; Laffont et al., 2002), it is likely that TCBZ would modulate its biliary or intestinal P-gp-mediated elimination.

The use of combinations of anthelmintics, each acting through different mechanisms, is thought to reduce the frequency of treatment and to delay the development of resistance (Barnes et al., 1995). Despite the fact that in vitro studies indicated that closantel modulated P-gp transport in cell lines (Dupuy et al., 2006), a similar plasma concentration profile was observed when closantel was co-administered with IVM and when each anthelmintic was used in isolation (Cromie et al., 2006). Alvarez et al. (2008) found an increase in IVM plasma concentrations when co-administered with ABZ to lambs and in the current study, the enhanced plasma concentrations of IVM in the plasma (87% higher AUC) after co-administration with TCBZ, indicates PK interaction.

Because of the complexity of transporter–enzyme and transporter–transporter interactions, it is difficult to extrapolate in vitro data on transporter-mediated drug interactions to in vivo settings (Lin, 2007). Interestingly, the outcome of the current in vivo experiments correlates with those of in vitro assays following the incubation of IVM in the presence of TCBZ. Several in vitro assays have been developed to assess the potential involvement of transporters in drug interactions. Caco-2 cell monolayers, derived from a human colonic adenocarcinoma, are widely used to estimate trans-epithelial passage of different P-gp substrates (Fricker et al., 1996). The use of everted gut sacs has been proposed as an accurate in vitro model of quantifying the P-gp-mediated intestinal efflux of different drugs (Barthe et al., 1998).

Recent in vitro work using this method demonstrated a marked increase in the IVM accumulation rate in the intestinal wall in the presence of P-gp modulators (Ballent et al., 2006). In the current trial the presence of TCBZ affected IVM intestinal transport. In the current experiment, the viability of the intestinal tissue was demonstrated by the glucose uptake assay. Overall, using this study system, the rate and extent of IVM accumulation in the intestinal wall was significantly enhanced by the presence of TCBZ.

The ability of different anthelmintics to interfere with P-gp transport in cell lines over-expressing P-gp has been studied by Dupuy et al. (2006). The presence of TCBZ and TCBZSO increased the intracellular concentration of rhodamine 123, a P-gp substrate with an inhibition constant (IC50) of 41 and 63 μM, respectively (Dupuy et al., 2006). Whereas TCBZ enhanced the intestinal accumulation of IVM in the current trial, the lower concentration of TCBZSO used (8 vs. 100 μM), when compared to the study by Dupuy et al. (2006) may explain the lack of effect observed following co-incubation with this metabolite.

Several changes were noted in the plasma concentration profile of TCBZ and its metabolites after its in vivo co-administration with IVM. The systemic availability of TCBZ was decreased and the C_{max} of the sulfoxide and sulfone metabolites was significantly enhanced after co-administration with IVM. Given that IVM, TCBZ and its metabolites are strongly bound to albumin in the peripheral circulation (Hennessy et al., 1987; Klotz et al., 1990), a drug binding displacement may occur when TCBZ and IVM are co-administered. Furthermore, as is the case for many drugs, significant interplay between transporter molecules and the cytochrome P-450 system may occur.

Transporter–enzyme interplay would complicate the interpretation of the underlying mechanisms of drug interactions (Lin, 2007). The involvement of the oxidative enzymatic systems, flavin-monooxygenase and cytochrome P-450, in TCBZ sulfoxidation and sulfonation in sheep was recently demonstrated in our laboratory (Virkel et al., 2006). As TCBZ may also interact with P-gp (Dupuy et al., 2006), the competition for P-gp transport observed following its co-administration with IVM may affect the rate and amount of TCBZ available to be metabolised during the first 12–24 h post-administration.

Supporting this hypothesis, the ratio of TCBZSO $AUC_{0–12\ h}$ to TCBZ $AUC_{0–12\ h}$ was significantly increased after co-administration of the flukicide and IVM. In consequence, a thorough understanding of the activities of P-gp and cytochrome P-450 is necessary when interpreting the pharmacokinetics of therapeutics for which these proteins act as substrates.

Conclusions

This study has elucidated, for the first time, the PK interactions of IVM and TCBZ in sheep following their IV administration. However, it is important to remember that such interactions do not always result in clinically significant effects (Fuhr, 2007). We have previously reported that IVM-modulation of P-gp activity decreases the efflux of TCBZ from resistant fluke and higher concentrations of TCBZ and TCBZSO were recovered from resistant fluke in the presence of IVM (Mottier et al., 2006). In the current study, the in vivo and in vitro IVM-TCBZ interactions provided further evidence of the clinical significance of this drug combination in parasite control. The role of hepatic and intestinal ABC transporters on the disposition and efficacy of different anthelmintic drug combinations is currently under evaluation in our laboratory.

Conflict of interest

None of the authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

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