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Involvement of P-glycoprotein on ivermectin kinetic behaviour in sheep: itraconazole-mediated changes on gastrointestinal disposition

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Different pharmacological approaches have been used in an attempt to increase the systemic availability of anthelmintic drugs. The comparative effect of the itraconazole (ITZ)-mediated modulation of P-glycoprotein (P-gp) activity on the *in vivo* kinetic behaviour of ivermectin (IVM) administered by the intravenous (i.v.) and intraruminal (i.r.) routes to sheep was assessed in the current work. Corriedale sheep received IVM (50 μg/kg) by the i.v. route either alone (group A) or co-administered with the P-gp modulator ITZ (100 mg orally three times every 12 h) (group B). Animals in groups C and D were intraruminally treated with IVM (50 μg/kg) alone or co-administered with ITZ (100 mg orally three times every 12 h) respectively. Jugular blood and gastrointestinal tissue samples (animals treated by the i.r. route) were collected. The samples were analysed by HPLC using fluorescence detection. The plasma disposition of IVM given intravenously was unaffected by the presence of ITZ. However, after the i.r. treatment the co-administration with ITZ resulted in markedly higher IVM plasma concentration profiles compared to the control group. Likewise, the presence of ITZ enhanced the IVM concentration profiles measured in the gastrointestinal mucosal tissues. An ITZ-induced reduction on the P-gp efflux activity at the intestinal lining may have accounted for the greater absorption and enhanced systemic availability observed for IVM in the intraruminally treated animals.

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INTRODUCTION

Ivermectin (IVM) is a macrocyclic lactone (ML) compound widely used as a broad-spectrum anthelmintic both in human and veterinary medicine. The high lipophilicity of IVM correlates with its extensive distribution to tissues of parasite location in ruminants (Lifschitz *et al.*, 2000). IVM is administered by different routes according to the target animal species, including subcutaneous, intramuscular, oral, intraruminal (i.r.) and topical routes. A lower IVM plasma availability after its i.r. administration to sheep compared with the parenteral treatment has been reported (Prichard *et al.*, 1985). This lower plasma concentration profiles obtained after the oral administration of IVM to sheep leads to poorer efficacy and shorter persistence of the antiparasitic activity compared with the subcutaneous treatment (Borgsteede, 1993). Although degradation of IVM (Ali & Hennessy, 1996) and doramectin (Hennessy *et al.*, 2000) in the ruminant gastrointestinal (GI) tract has not been ruled out, a high metabolic stability of IVM, not only in sheep ruminal content but also in the acid environment of the abomasal content, has been reported after 24 h of incubation (Lifschitz *et al.*, 2005). The association of orally administered IVM with the particulate phase of the GI content in sheep fed on a lucerne/wheaten (50:50) ration (Ali & Hennessy, 1996) or *ad libitum* with lucerne hay (Lifschitz *et al.*, 2005) has been suggested as one of the main cause of the difference observed between administration routes in terms of drug systemic availability.

P-glycoprotein (P-gp) is a transmembrane protein associated with a phenotype of multidrug resistance (MDR) to certain anticancer drugs in mammalian cancer cells, which is able to pump a broad range of structurally and functionally unrelated compounds out of the cell by an ATP-dependent process (Lin, 2003). P-gp is physiologically expressed in a number of tissues including liver, blood–brain barrier, placenta and intestine (Thiebaut *et al.*, 1987). At the intestinal level, P-gp may act by limiting the absorption of orally administered drugs (Watkins, 1997). IVM has been reported as a P-gp substrate (Schinkel *et al.*, 1994, 1996; Poulliot *et al.*, 1997) and the role of P-gp on

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the GI secretion of IVM has been demonstrated using the intestinal closed-loop model in rats (Laffont *et al.*, 2002). Recent *in vitro* work carried out using the everted sac technique demonstrated a marked increase on the IVM accumulation rate in the ileum wall in the presence of itraconazole (ITZ) (Ballent *et al.*, 2006). Itraconazole is a fungistatic/fungicidal agent widely used in human and veterinary medicine and has been described as a potent P-gp and cytochrome P450 3A (CYP3A) inhibitor (Cooper *et al.*, 2003). As previous reports have demonstrated that IVM is largely excreted in bile and faeces as the unchanged parent drug (Chiu *et al.*, 1990; Lifschitz *et al.*, 2000), it is likely that the inhibition of CYP3A by ITZ would only play a minor role in the IVM pharmacokinetic profile when it is co-administered with the antifungal compound.

To achieve further comprehension of the involvement of the P-gp efflux activity on the IVM GI disposition in sheep, the experimental goal of the current study was to assess the comparative effect of the ITZ-mediated modulation of P-gp activity on the *in vivo* kinetic behaviour of IVM administered by intravenous (i.v.) and i.r. routes to sheep.

MATERIALS AND METHODS

Experiment 1: 'Intravenous treatment'

The study was conducted in clinically healthy and parasite-free sheep. Twenty Corriedale (25–30 kg) sheep were used in this trial. The animals were kept under field conditions during the whole experimental period. The health of the animals was monitored prior to and throughout the experimental period. Animals were in optimal nutritional condition, grazing on a lucerne/red clover pasture during the whole experimental period with free access to water.

Experimental animals were randomly allocated into two groups of 10 sheep each. Animals in group A (IVM alone) received IVM by i.v. injection at 50 μg/kg (Ivosint®; Biogénesis, Buenos Aires, Argentina). The original formulation was diluted in propylene glycol (1:4) to fit the low dose volumes. Sheep in group B (IVM + ITZ) were treated with IVM (i.v. injection, 50 μg/kg) co-administered with ITZ (Itrac®; Pablo Cassará, Buenos Aires, Argentina) (100 mg orally three times every 12 h). Jugular blood was collected into heparinized vacutainer tubes at 10, 20, 30, 60 min and 2, 4, 8, 12, 24, 36, 48 h and 3, 4, 5, 7, 9, 11, 15 days post-treatment. Blood samples were centrifuged at 2000 ***g*** for 20 min. The recovered plasma was kept in labelled vials and stored at −20 °C until analysed by HPLC.

Experiment 2: 'Intraruminal treatment'

Twenty-four Corriedale sheep weighing 25–30 kg were used in this experiment. Animals were kept in the same conditions than in the experiment 1. Sheep were randomly allocated into two groups of 12 animals each. Treatments were given as follows:

Group C (IVM alone): experimental animals were treated with IVM (Ivosint®; Biogénesis) by i.r. injection at 50 μg/kg. The original formulation was diluted in propylene glycol (1:4) to fit the low dose volumes. Group D (IVM + ITZ): animals were treated with IVM (i.r. injection at 50 μg/kg) co-administered with ITZ (Itrac®; Pablo Cassará) (100 mg orally three times every 12 h). Blood samples were taken at 30, 60 min and 2, 4, 8, 12, 24, 36, 48 h and 3, 4, 5, 7, 9, 11, 15 days post-treatment. Additionally, 12 IVM intraruminally treated sheep with (group D) and without (group C) ITZ were killed at 1 and 3 days post-treatment by a captive bolt and immediately exsanguinated according to the institutional and international animal euthanasia guidelines [Animal Welfare Act. Academic Council Resolution 087/02, Faculty of Veterinary Medicine (UNCPBA), American Veterinary Medical Association, 2001]. Samples collected included liver, bile, mucosal tissue and luminal content of the GI tract. The GI tract was sampled at five different sections: abomasum, duodenum, jejunum, ileum and colon. After collection of the intestinal and abomasal contents, the mucosal tissues of each GI section were obtained by scraping. Both duodenal content and mucosa were collected before the bile duct opening. Bile was collected directly from the gall bladder on killing. All samples were transported on ice to the laboratory and processed as described for Experiment 1.

Analytical procedures

The extraction of IVM from experimental plasma and GI samples was carried out following the technique earlier described by Alvinerie *et al.* (1993) and adapted by Lifschitz *et al.* (2000). Samples of plasma, liver tissue, bile and intestinal mucosal tissues and luminal contents (0.250 mL or g) were fortified with 20 ng/g or mL of abamectin (ABM) (used as internal standard, 10 ng/10 μL) and 0.25 mL of acetonitrile plus 70 μL of deionized water were added to each sample. The preparation was mixed (Multi Tube Vortexer; VWR Scientific Products, West Chester, PA, USA) over 15 min, sonicated in an ultrasonic bath for 10 min (Transsonic 570/H; Laboratory Line Instruments Inc., Melrose Park, IL, USA) and the solvent–sample mixture was centrifuged at 2000 ***g*** for 10 min. The supernatant was manually transferred into a tube and the procedure was repeated once for the liver and intestinal tissue samples. The supernatant was applied to a conditioned Supelclean LC 18 cartridge (Supelco, Bellefonte, PA, USA). After washing with 1 mL deionized water followed by 1 mL water–methanol (4:1 v/v), the cartridges were dried off for 5 min and the sample eluted with 1.5 mL methanol, and was collected. After solid phase extraction, the elute was evaporated to dryness under a gentle stream of dry nitrogen at 60 °C in a water bath. The sample was subjected to a derivatization described by De Montigny *et al.* (1990). After completion of the reaction, an aliquot (100 μL) of each sample was injected directly into the chromatographic system.

Ivermectin analysis and validation procedures

The measurement of IVM concentrations in plasma and tissues was carried out using an HPLC (Shimadzu 10A HPLC system;

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Shimadzu Corp., Kyoto, Japan). The HPLC analysis was performed following the technique described by Lifschitz *et al.* (2000). The chromatographic conditions included a mobile 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, Torrance, CA, USA) (5 μm, 4.6 mm × 250 mm). IVM detection was performed using a fluorescence detector (Spectrofluorometric detector RF-10; Shimadzu, Kyoto, Japan) 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 IVM concentration in spiked (validation of the analytical method) and experimental samples. There was no interference of endogenous compounds in the chromatographic determinations. The analytical procedures, including chemical extraction and HPLC analysis of IVM in plasma, liver, bile and intestinal mucosa and luminal contents were validated. The statistical program (Instat 3.0; Graph Pad Software Inc., San Diego, CA, USA) 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, 0.5 and 100 ng/g for GI mucosal tissue and 1 and 100 ng/g for liver tissue, bile and luminal content. Linearity was established to express the concentration-detector response relationship, as determined by injection of plasma and tissues IVM spiked standards at different concentrations (three replicates). Calibration curves were established using least squares linear regression analysis and correlation coefficients (r) and coefficient of variations (CV) were calculated. Drug recovery was estimated by comparison of 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 higher than 70% and a CV <20%. The inter-assay precision of the analytical procedures obtained after HPLC analysis of IVM spiked standards (1 ng and 40 ng/mL or ng/g) on different working days, showed CV between 1.8% and 3.5%. The linear regression lines for all tissues analysed showed correlation coefficients ≥0.998. The mean recoveries of IVM were in a range between 73% and 98% (plasma), 81% and 96% (liver), 86% and 89% (bile), 83% and 94% (mucosal tissue) and 81% and 99% (luminal content). The limit of quantification was established at 0.2 ng/mL for plasma, 0.5 ng/g for GI mucosal tissue and 1 ng/g for liver, bile and GI luminal content.

Pharmacokinetic and statistical analyses of the data

The plasma and GI tissue concentrations vs. time curves obtained after each treatment in each individual animal were fitted with the PK Solutions 2.0 (Ashland, OH, USA) computer software. Pharmacokinetic parameters were determined using a noncompartmental model method. For the i.r. administration of IVM, the peak concentration (C_{max}) and time to peak concentration (t_{max}) were read from the plotted concentration–time curve in each individual animal. The terminal (elimination) half-life ($t_{1/2el}$) and absorption half-life ($t_{1/2ab}$) were calculated as ln $2/\lambda_z$ and ln $2/k_{ab}$, respectively, where λ_z is the elimination rate constant and k_{ab} represents the first order absorption rate constant. The area under the concentration–time curves (AUC) were calculated by the trapezoidal rule (Gibaldi & Perrier, 1982) and further extrapolated to infinity by dividing the last experimental concentration by the terminal slope (λ_z). Statistical moment theory was applied to calculate the mean residence time (MRT) for IVM as follows:

$$MRT = \frac{AUMC}{AUC},$$

where AUC is as defined previously and $AUMC$ is the area under the curve of the product of time and drug concentration vs. time from zero to infinity (Gibaldi & Perrier, 1982). The volume of distribution ($V_{d(area)}$) was estimated according to the following equation:

$$V_{d(area)} = \frac{\text{Dose}}{AUC \cdot \lambda_z}$$

and the total body clearance (Cl_B) was calculated by

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

Ivermectin plasma, bile and GI tissue concentrations and all the estimated pharmacokinetic parameters are reported as mean ± SEM. A normality test was performed for testing if the data are sampled from populations that follow Gaussian distributions. This assumption was tested using the Kolmogorov and Smirnov method. Mean pharmacokinetic parameters for IVM obtained after its administration either alone or co-administered with ITZ were statistically compared using Student's t-test. The assumption that the data obtained after both treatments have the same variance was assessed. A nonparametric Mann–Whitney test was used where significant differences among standard deviations were observed. A value of $P < 0.05$ was considered statistically significant.

RESULTS

The work reported here investigates the *in vivo* effect of intestinal P-gp modulation on the IVM kinetic behaviour following its administration by i.v. and i.r. routes to sheep. IVM was recovered from the bloodstream between 10 min and 11 days (i.v.) and from 30 min and 15 days (i.r.) post-treatment. Additionally, IVM was recovered in the GI tissues under analysis at 1 and 3 days post-treatment.

The plasma disposition of IVM given intravenously was unaffected by the presence of ITZ (Fig. 1). There were no statistically significant differences in AUC values for IVM after its administration with or without ITZ. The pharmacokinetic parameters obtained for IVM in plasma after both treatments are summarized in Table 1. However, the ITZ modulation of the P-gp intestinal activity was clearly observed following the i.r. administration of the antiparasitic compound. The co-administration with ITZ resulted in markedly higher IVM plasma concentration profiles compared with the control group (IVM

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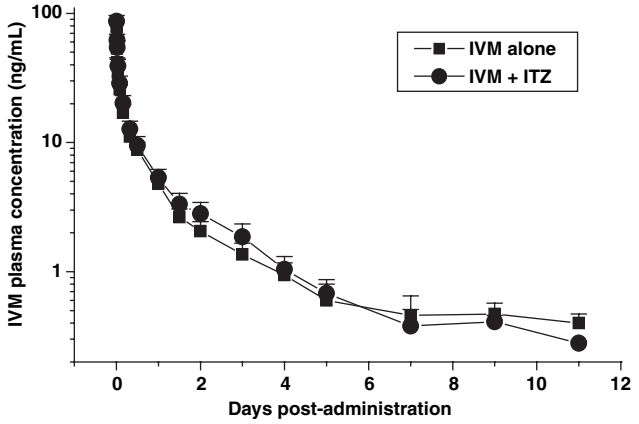

Fig. 1. Mean (±SEM) ($n = 6$) ivermectin (IVM) plasma concentrations (ng/mL) obtained after its intravenous administration (50 μg/kg) either alone or co-administered with itraconazole (ITZ) (100 mg orally three times every 12 h) to sheep.

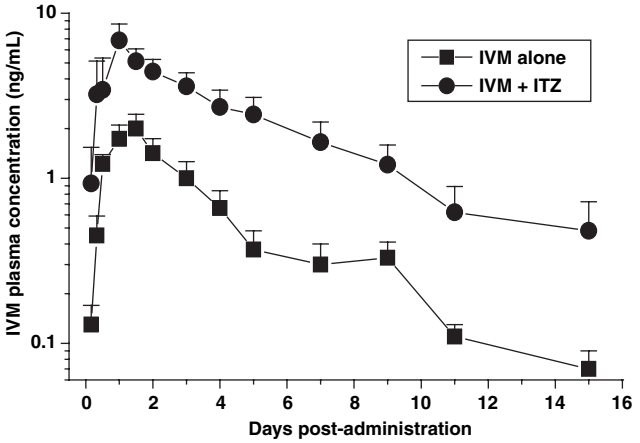

Fig. 2. Mean (±SEM) ($n = 6$) ivermectin (IVM) plasma concentrations (ng/mL) obtained after its intraruminal administration (50 μg/kg) either alone or co-administered with itraconazole (ITZ) (100 mg orally three times every 12 h) to sheep.

Table 1. Mean (±SEM) ($n = 6$) kinetic parameters for ivermectin (IVM) in plasma obtained after its intravenous administration (50 μg/kg) either alone or co-administered with itraconazole (ITZ) (100 mg orally three times every 12 h) to sheep

Kinetic parameters	IVM alone	IVM + ITZ
AUC_{total} (ng·day/mL)	16.5 ± 2.82	17.4 ± 1.64
$AUMC_{total}$ (ng·day/mL)	17.1 ± 3.03	20.1 ± 2.07
$t_{1/2el}$ (day)	0.91 ± 0.03	1.02 ± 0.07
MRT (day)	1.03 ± 0.02	1.17 ± 0.08
$V_{d(area)}$ (L/kg)	4.74 ± 0.87	4.52 ± 0.77
Cl_B (L/kg·day)	3.54 ± 0.59	3.01 ± 0.33

AUC_{total}, area under the concentration vs. time curve extrapolated to infinity; $AUMC_{total}$, area under the first-moment concentration vs. time curve extrapolated to infinity; $t_{1/2el}$, elimination half-life; *MRT*, mean residence time; $V_{d(area)}$, volume of distribution; Cl_B, total body clearance.

Table 2. Mean (±SEM) ($n = 6$) kinetic parameters for ivermectin (IVM) in plasma obtained after its intraruminal administration (50 μg/kg) either alone or co-administered with itraconazole (ITZ) (100 mg orally three times every 12 h) to sheep

Kinetic parameters	IVM alone	IVM + ITZ
$t_{1/2ab}$ (day)	0.23 ± 0.04	0.35 ± 0.06
t_{max} (day)	1.25 ± 0.17	1.00 ± 0.00
C_{max} (ng/mL)	2.09 ± 0.41	6.85 ± 1.76*
AUC_{total} (ng·day/mL)	6.95 ± 1.71	26.5 ± 7.31*
$AUMC_{total}$ (ng·day/mL)	26.5 ± 8.10	144 ± 56.1*
$t_{1/2el}$ (day)	2.10 ± 0.33	2.76 ± 0.50
MRT (day)	3.46 ± 0.51	4.54 ± 0.73

$t_{1/2ab}$, absorption half-life; t_{max}, time to peak plasma concentration; C_{max}, peak plasma concentration; AUC_{total}, area under the concentration vs. time curve extrapolated to infinity; $AUMC_{total}$, area under the first-moment concentration vs. time curve extrapolated to infinity; $t_{1/2el}$, elimination half-life; *MRT*, mean residence time.
*Mean kinetic parameters are significantly different from those obtained for the IVM alone treatment at $P < 0.05$.

alone). The IVM plasma concentration profiles measured up to 15 days after its i.r. administration alone and co-administered with ITZ to sheep is compared in Fig. 2.

The higher IVM plasma concentrations measured after its i.r. co-administration with ITZ accounted for the observed increased drug systemic availability. The *AUC* values obtained after i.r. administration of IVM was 3.8-fold higher in the presence of ITZ suggesting an enhancement in the intestinal absorption of the compound. Furthermore significantly higher peak plasma concentrations (C_{max}) were measured after the IVM + ITZ treatment compared with the i.r. administration of IVM alone. Table 2 shows the pharmacokinetic parameters for IVM after its i.r. administration alone or co-administered with ITZ.

Likewise, the presence of ITZ enhanced the IVM concentration profiles measured in different GI mucosal tissues after its co-administration with ITZ. The percentage of IVM concentration enhancement compared with the control group in abomasum, duodenum, jejunum, ileum and colon obtained after the co-administration with the P-gp modulator agent is shown in Fig. 3. The ratios between IVM concentrations measured in the luminal content and the mucosal tissue of the different GI tract segments was used as an estimator of the modulation of the P-gp-mediated intestinal secretion process. The comparative ratios between IVM concentration in the luminal content and in the mucosal tissue in the different intestinal sections after both treatments are shown in Fig. 4. The high variability in the IVM concentration data in liver and bile account for the lack of statistical differences between the control and ITZ-coadministered groups. However differences on IVM disposition were observed in liver tissue and bile, where the IVM concentrations were 48% and 213% higher in the IVM + ITZ co-administration group compared with IVM alone group (Fig. 5). There was no changes in the ratio between IVM concentrations in liver and bile after its administration alone (0.61 ± 0.11) or co-administered with the P-gp modulator (0.46 ± 0.06).

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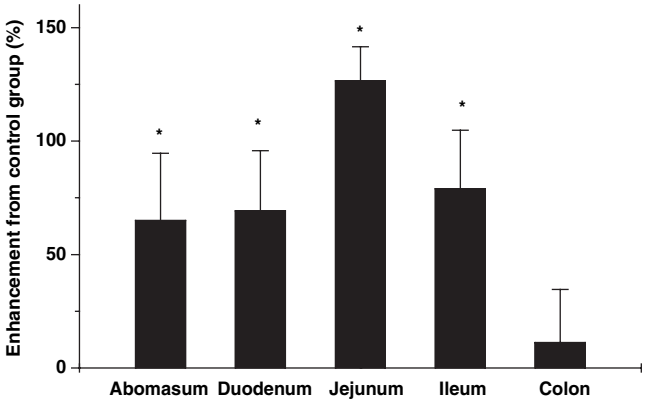

Fig. 3. Enhancement of the ivermectin (IVM) concentration profiles measured in the wall tissue of different gastrointestinal sections after its intraruminal administration (50 μg/kg) co-administered with ITZ (100 mg orally three times every 12 h) to sheep. The results (mean ± SEM, $n = 6$) are expressed as the percentage of enhancement compared with the control group (IVM alone). Values are statistically different from those obtained after the IVM alone treatment at $^*P < 0.05$.

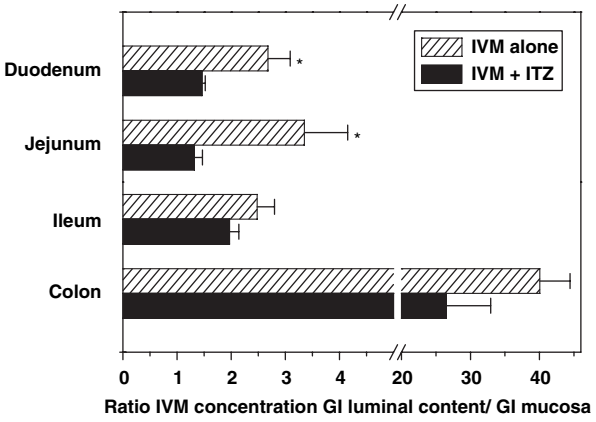

Fig. 4. Ratio between ivermectin (IVM) concentrations (mean ± SEM, $n = 6$) measured in the luminal content and mucosal tissue of different gastrointestinal (GI) sections after its intraruminal administration (50 μg/kg) either alone or co-administered with itraconazole (ITZ) (100 mg orally three times every 12 h) to sheep. Values are statistically different from those obtained after the IVM + ITZ treatment at $^*P < 0.05$.

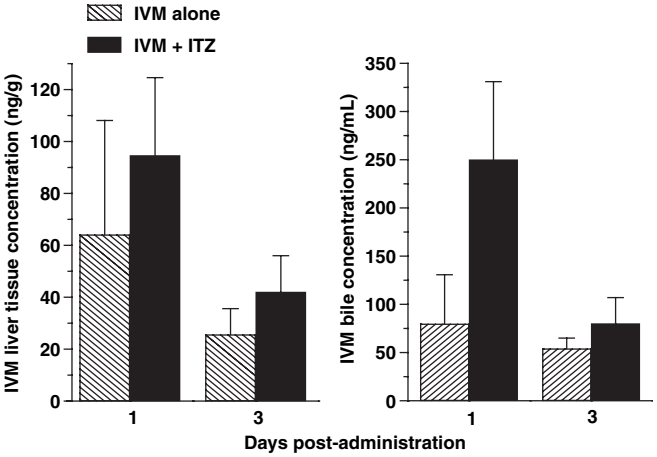

Fig. 5. Effects of itraconazole (ITZ) (100 mg orally three times every 12 h) on the concentration of ivermectin (IVM) (50 μg/kg intraruminal injection) in liver tissue and bile after their co-administration in sheep.

DISCUSSION

Different pharmacological approaches have been used in an attempt to increase systemic availability of anthelmintic drugs in veterinary medicine. The main purpose of the study of the interaction between P-gp substrates and P-gp modulators was to identify the pharmacokinetic consequences and to predict the clinical outcome. *In vitro* assays performed with tumour cell lines showed that IVM inhibited P-gp activity and restored the sensitivity to antitumour compounds (Didier & Loor, 1996; Poulliot *et al.*, 1997). The *in vivo* co-administration of ML with P-gp modulators has been proposed as a strategy to enhance drug systemic availability and to increase the anthelmintic efficacy (Lifschitz *et al.*, 2002, 2004; Dupuy *et al.*, 2003; Molento *et al.*, 2004). The presence of loperamide (LPM), used as a P-gp modulator, induced changes to the plasma disposition and pattern of faecal excretion of the ML compound moxidectin, given both intravenously and subcutaneously to cattle (Lifschitz *et al.*, 2002). Likewise, co-administration of IVM with LPM resulted in changes to the pattern of IVM bile-faecal excretion in rats, which accounted for an enhanced availability of the antiparasitic compound in tissues of parasite location (Lifschitz *et al.*, 2004). Whereas IVM given alone had a 0% reduction in the number of parasite eggs in sheep faeces, a 75% reduction in the egg faecal counts was achieved after the co-administration of IVM with verapamil, a P-gp substrate compound (Borges *et al.*, 2005). In the current trial, the presence of ITZ did not induce any marked change on the plasma kinetic of IVM administered by the i.v. route to sheep (Fig. 1). The IVM plasma *AUC* values ranged between 22.5 (IVM alone) and 25.3 ng·d/mL (IVM + ITZ) (Table 1). The total body clearance was slightly lower (17%) when ITZ was co-administered. A clinically relevant drug interaction after the concomitant administration of two different compounds may occur if adequate concentrations are in the site of action at the same time. Although there are no comparative data on the pharmacokinetics of IVM and ITZ in ruminants, a faster disposition of ITZ was observed in rats (elimination half-life: 9 h) (Shin *et al.*, 2004) compared to IVM (elimination half-life: 30 h) (Lifschitz *et al.*, 2006). This faster disposition and the higher plasma concentrations of IVM during the first 2 days postadministration may have accounted for the lack of interaction between both compounds after the i.v. injection of the endectocide molecule.

On the other hand, the i.r. administration of IVM co-administered with ITZ resulted in markedly higher IVM plasma profiles compared with the IVM alone treatment (Fig. 2). The higher IVM plasma concentrations measured in the presence of ITZ accounted for a significant increment on the *AUC* and C_{max} values (Table 2). A greater enhancement in the C_{max} (227%) and *AUC* (280%) values was obtained in the current trial with

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the co-administration of ITZ compared with those obtained where verapamil was used as P-gp modulator (83% and 53% respectively) (Molento *et al.*, 2004). The lower dose rate of IVM used in the current work that increased the molar ratio between the P-gp modulator and IVM and a more efficient ITZ-mediated modulation of P-gp activity may explain the obtained differences between both trials in sheep. An ITZ-induced reduction on the P-gp efflux activity at the intestinal lining of the small intestine may have accounted for the greater absorption and enhanced systemic availability observed for IVM in the intraruminally treated animals. Therefore, it is likely that the pharmacological implications of the *in vivo* interaction between P-gp and IVM may differ according to the route of IVM administration.

The oral administration of the ML to sheep has been correlated with a low systemic availability (Marriner *et al.*, 1987; Imperiale *et al.*, 2004; Lespine *et al.*, 2004) and shorter persistence of the antiparasitic activity (Borgsteede, 1993) compared with the subcutaneous treatment. As the stability of IVM in ruminal fluid was demonstrated in previous reports (Bogan & McKellar, 1988; Andrew & Halley, 1996; Lifschitz *et al.*, 2005) the high association of IVM to the particulate material of digesta appears as a relevant factor modulating the ML absorption process. Significantly higher concentrations (between 90% and 99% of the total drug recovered) of IVM and moxidectin were measured in the solid particulate phase of ruminal and abomasal contents compared with the fluid phase (Ali & Hennessy, 1996; Lifschitz *et al.*, 2005). The influence of the P-gp-mediated intestinal secretion on the bioavailability of intraruminally administered IVM was corroborated in the current work. The differential effect induced by ITZ co-administered with IVM given either by the i.r. or the i.v. route may be explained by the singular partition of this drug in the GI tract. The low amount of free drug available in the GI lumen to be absorbed, following the oral/i.r. administration of IVM, may favour the P-gp-mediated GI efflux which could contribute to its limited systemic availability. Thus, the presence of P-gp modulator agent ITZ induced a significant increment on the IVM absolute bioavailability obtained after its i.r. administration to sheep.

Higher GI tissue concentrations of IVM (between 11% and 126%) were observed in the presence of the P-gp modulator agent compared with the control group (Fig. 3). In spite of the fact that the current experimental design was not addressed to study the regional differences in P-gp expression along the GI tract, useful information may be obtained. The greatest enhancement on IVM concentrations obtained after its co-administration with ITZ was in the jejunum. Stephens *et al.* (2002) showed that P-gp expression was higher in ileum and distal colon in mice. Using an intestinal closed-loop model, the greatest intestinal elimination of IVM in rats occurs in the jejunum segment (Laffont *et al.*, 2002). The relationship between IVM disposition in GI mucosal tissue and luminal content could be useful to assess the influence of the modulation on the P-gp secretion activity. The ratios of drug concentrations measured in the luminal content/mucosal tissue tended to be lower in the co-administered sheep compared with the control group. The highest reduction value in the current study was obtained in the jejunum (61%) (Fig. 4). Similarly, the greatest verapamil-induced inhibition of IVM intestinal secretion in rats was observed in the jejunum (Laffont *et al.*, 2002). Further studies are needed to clarify the inter species differences on the P-gp expression and activity along the GI tract which may have considerable physio-pharmacological relevance in veterinary medicine.

Although without reaching statistical differences, the ITZ-mediated modulation of P-gp activity facilitated the increment of IVM concentration in liver tissue and bile (Fig. 5). However, the ratio between IVM concentrations in liver and bile was similar in both experimental groups suggesting that the observed increase in the IVM liver availability was largely due to an inhibitory effect on the P-gp mediated intestinal secretion. Laffont *et al.* (2002) reported that intestinal secretion is the major route of parent IVM elimination in the rat. The relative involvement of the biliary and intestinal excretion mechanisms for a P-gp substrate, such as IVM, in ruminants needs to be elucidated. Considering the observed enhancement of IVM systemic availability induced by ITZ and the well-established correlation between ML plasma profiles and those achieved at the tissues of parasite location (Lifschitz *et al.*, 1999, 2000), the use of P-gp modulators could be a further step on the search of pharmacological tools addressed to optimize drug use in veterinary medicine in the face of the worldwide development of anthelmintic resistance. *In vivo* co-administration trials and *in vitro* permeation studies with the Ussing chamber system are currently being performed in our laboratory to establish the involvement of intestinal ABC transporters on the disposition of anthelmintic drugs in ruminants.

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