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Cattle nematodes resistant to macrocyclic lactones: Comparative effects of P-glycoprotein modulation on the efficacy and disposition kinetics of ivermectin and moxidectin

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ABSTRACT

The role of the drug efflux pump, known as P-glycoprotein, in the pharmacokinetic disposition (host) and resistance mechanisms (target parasites) of the macrocyclic lactone (ML) antiparasitic compounds has been demonstrated. To achieve a deeper comprehension on the relationship between their pharmacokinetic and pharmacodynamic behaviors, the aim of the current work was to assess the comparative effect of loperamide, a well-established P-glycoprotein modulator, on the ivermectin and moxidectin disposition kinetics and efficacy against resistant nematodes in cattle. Fifty (50) Aberdeen Angus male calves were divided into five (5) experimental groups. Group A remained as an untreated control. Animals in the other experimental Groups received ivermectin (Group B) and moxidectin (Group C) (200 μg/kg, subcutaneuosly) given alone or co-administered with loperamide (0.4 mg/kg, three times every 24 h) (Groups D and E). Blood samples were collected over 30 days post-treatment and drug plasma concentrations were measured by HPLC with fluorescence detection. Estimation of the anthelmintic efficacy for the different drug treatments was performed by the faecal egg count reduction test (FECRT). Nematode larvae were identified by pooled faecal cultures for each experimental group. *Cooperia* spp. and *Ostertagia* spp. were the largely predominant nematode larvae in pre-treatment cultures. A low nematodicidal efficacy (measured by the FECRT) was observed for both ivermectin (23%) and moxidectin (69%) in cattle, which agrees with a high degree of resistance to both molecules. *Cooperia* spp. was the most abundant nematode species recovered after the different drug treatments. The egg output reduction values increased from 23% to 50% (ivermectin) and from 69% to 87% (moxidectin) following their co-administration with loperamide. Enhanced systemic concentrations and an altered disposition of both ML in cattle, which correlates with a tendency to increased anthelmintic efficacy, were observed in the presence of loperamide. Overall, the *in vivo* modulation of P-glycoprotein activity modified the kinetic behavior and improved the efficacy of the ML against resistant nematodes in cattle. The work provides further evidence on the high degree of resistance to ML in cattle nematodes and, shows for the first time under field conditions, that modulation of P-glycoprotein may be a valid pharmacological approach to improve the activity and extend the lifespan of these antiparasitic molecules.

1. Introduction

The control of nematode infections in livestock relies mainly on chemotherapy. The macrocyclic lactones (ML) are one of the major classes of anthelmintics available for treatment of parasitic diseases. The ML compounds such as ivermectin (IVM) and moxidectin (MXD), exhibit a broad-spectrum of activity against gastrointestinal and lung nematodes as well as ectoparasites of domestic animals (McKellar and Benchaoui, 1996). The anthelmintic resistance (AR) to ML was firstly recognized as a problem in small ruminant production systems (Prichard, 1994). However, several reports of anthelmintic resistance in bovine gastro-intestinal nematodes have been recently reported in Argentina and other countries (Fiel et al., 2001; Familton et al., 2001; Anziani et al., 2004; Stafford et al., 2007; Demeler et al., 2009).

P-glycoprotein (P-gp) is a transmembrane protein associated with a phenotype of multidrug resistance to certain anticancer drugs

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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. P-gp is physiologically expressed in a number of tissues, including liver, blood–brain barrier, and intestine (Lin, 2003). The ML compounds have been reported to behave as P-gp substrates (Schinkel et al., 1994; Didier and Loor, 1995, 1996; Pouliot et al., 1997). It has also been shown that affinity by P-gp may differ among different ML molecules (Lespine et al., 2007).

IVM and MXD are largely excreted in bile and faeces as the unchanged parent drugs in cattle (Lifschitz et al., 1999a, 2000). The role of P-gp on the gastrointestinal secretion of IVM has been demonstrated *in vitro* using the intestinal closed-loop model and the everted sac technique in rats (Laffont et al., 2002; Ballent et al., 2006). Different experiments have been carried out to study the drug–drug interaction of ML with different chemical substances known as P-gp modulators. The P-gp modulator agents seem to inhibit P-gp activity by competing with the P-gp binding site and/or through inhibition of ATP hydrolysis (Garrigos 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. The presence of loperamide (LPM), used as a P-gp modulator, induced changes to the plasma disposition and pattern of faecal excretion of MXD, when both were concomitantly given intravenously or 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).

The involvement of some of the ABC transporters, such as P-gp, in the genetic changes associated with ML pharmacodynamic mechanisms of resistance in nematodes was previously reported (Xu et al., 1998; Prichard and Roulet, 2007). The aim of the current work was to assess the comparative effect of loperamide, a well-established P-gp modulator, on the IVM and MXD disposition kinetics and efficacy against resistant nematodes in cattle. The achievement of a deeper comprehension on the relationship between the pharmacokinetics (host) and pharmacodynamics (target nematode) for the ML compounds is critical to prolong their lifespan as a major class of anthelmintic drugs in livestock.

2. Materials and methods

2.1. Animals

The study was conducted in a cattle ranch located in the west of the Buenos Aires Province, Argentina, with a well characterized history of resistance to ML. Previous evaluation of efficacy during two consecutive years by faecal egg count reduction test showed a reduction of 15 and 34.5% after IVM treatment. Faecal culture showed that *Cooperia oncophora* was the main resistant nematode involved (Suarez et al., unpublished data). Fifty (50) Aberdeen Angus male calves (236.3 ± 21.2 kg b.w.) naturally infected with IVM resistant nematodes were used in this trial. The selection of the animals was based on worm egg per gram counts (epg). On day −1, all calves were checked for epg, ear tagged and the individual body weights were recorded. Experimental animals had an average of 476 (±542) epg counts (range: 60–3160). 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).

2.2. Experimental design, treatments and samplings

Experimental calves were assigned into five (5) experimental groups. Group A remained as untreated control. Other animals received ivermectin (Bagomectina®, Laboratorios Bago Argentina) (Group B) or moxidectin (Cydectin®, Fort-Dodge Argentina) (Group C) (200 μg/kg, subcutaneuosly) either alone or co-administered with loperamide (0.4 mg/kg, repeated three times every 24 h by subcutaneous injection) compounded in a propylene glycol–DMSO solvent mixture (90:10) (Groups D and E, respectively). LPM was administered at a therapeutic dose rate, administered three times every 24 h to cover a 72-h period of the respective ML plasma profile. No adverse side effects induced by LPM were observed. Faecal samples were obtained from all calves of each experimental group at 0 and 14 days post-treatment in order to estimate the clinical efficacy of each drug treatment. Jugular blood samples (7 ml) were collected into heparinised vacutainer tubes prior to and at 0.33, 1, 2, 3, 8, 14, 21 and 30 days post-treatment. Blood samples were centrifuged at 2000*g* for 20 min and the recovered plasma was kept in labeled vials. Plasma samples were stored at −20 °C until analyzed by high performance liquid chromatography (HPLC).

3. Analytical procedures

3.1. Parasitological techniques

The anthelmintic efficacy of each drug treatment was estimated by the faecal egg count reduction test (FECRT). Faecal samples were obtained from all calves of each group at 0 and 14 days post-treatment and individual egg counts were performed using the modified McMaster technique (Roberts and O'Sullivan, 1949). For third-stage larvae differentiation, 2 g faecal samples were taken from each animal for faecal culture (Suarez, 1997). Larvae genera percentages were obtained from the mean of four pools per experimental group. For each treated group FECRT was determined according to the World Association for the Advancement of Veterinary Parasitology (WAAVP) recommendation using the formula (Coles et al., 1992):

FECRT (%) = $100 \times (1 - T_2/C_2)$, where T_2 is the arithmetic mean epg counts in the treated group at 14 days post-treatment and C_2 is the arithmetic mean epg counts in the untreated control group at 14 days post-treatment. Additionally, the FECRT was calculated using the modification of Abbott's formula (Henderson and Tilton, 1955) that was proposed by Dash et al. (1988): FECRT (%) = $(1 - T_2/T_1 \times C_1/C_2) \times 100$; where T_1 and T_2 are the arithmetic mean epg counts in the treated group at day 0 and day 14, respectively, and C_1 and C_2 are the arithmetic mean epg counts in the control group at day 0 and day 14, respectively.

3.2. Pharmacological determinations

3.2.1. IVM and MXD chemical extraction and derivatization

The extraction of IVM and MXD from spiked and experimental plasma samples was carried out following the technique first described by Alvinerie et al. (1993, 1995), slightly modified by Lifschitz et al. (1999b, 2002). Basically, 1-ml aliquot of plasma sample was combined with 10 ng of the internal standard compound (abamectin) and then mixed with 1 ml of acetonitrile–water (4:1). After mixing for 20 min, the solvent-sample mixture was centrifuged at 2000*g* during 15 min. The supernatant was manually transferred into a tube that was then placed on the appropriate rack of an Aspec XL sample processor (Gilson, Villiers Le Bel, France). The supernatant was injected to Strata C18-T cartridge (Phenomenex, Torrance, CA, USA), previously conditioned by passing 2 ml methanol and 2 ml deionized water. The cartridge was flushed with 1 ml of water and 1 ml of water/methanol (4:1). The compounds were eluted with 1.5 ml of methanol and concentrated to dryness under a stream of nitrogen. The re-suspension was done with 100 μl of a solution of *N*-methylimidazole (Sigma Chemical,

St. Louis, MO, USA) in acetonitrile (1:1) (De Montigny et al., 1990). Derivatization was initiated adding 150 μl of trifluoroacetic anhydride (Sigma Chemical, St. Louis, MO, USA) solution in acetonitrile (1:2). After completion of the reaction (<30 s), an aliquot (100 μl) of this solution was injected directly into the chromatograph.

3.2.2. Chromatographic conditions

IVM and MXD concentrations were determined by HPLC using a Shimadzu 10 A HPLC system with autosampler (Shimadzu Corporation, Kyoto, Japan). HPLC analysis was undertaken using a reverse phase C_{18} column (Kromasil, Eka Chemicals, Bohus, Sweden, 5 μm, 4.6 mm × 250 mm) and an acetic acid 0.2% in water/methanol/acetonitrile (0.5/60/39.5) mobile phase at a flow rate of 1.5 ml/min at 30 °C. IVM and MXD were detected with a fluorescence detector (Shimadzu, RF-10 Spectrofluorometric detector, Kyoto, Japan), reading at 365 nm (excitation) and 475 nm (emission wavelength). IVM and MXD concentrations were determined by the internal standard method using the Class LC 10 Software version 1.2 (Shimadzu Corporation, Kyoto, Japan). The peak area ratios were considered to calculate drug concentrations in spiked (validation) and experimental plasma samples. There was no interference of endogenous compounds in the chromatographic determinations. The solvents (Baker, Phillipsburg, NJ, USA) used during the extraction and drug analysis were HPLC grade. A complete validation of the analytical procedures used for extraction and quantification of IVM and MXD was performed before starting analysis of the experimental samples obtained during the pharmacokinetic trial. Calibration curves in the range between 0.2 and 100 ng/ml were prepared for each compound. Calibration curves were established using least squares linear regression analysis and correlation coefficients (r) and coefficient of variations (CV) calculated. Linearity was established to determine the IVM concentrations/detector responses relationship. Percentages of IVM and MXD recovery from plasma were obtained in the range between 0.2 and 100 ng/ml. The precision of the extraction and chromatography procedures was estimated by processing replicate aliquots ($n = 4$) of pooled cattle plasma samples containing known IVM and MXD concentrations (0.2, 10 and 50 ng/ml) on different working days. The limit of quantification was established as the lowest concentration measured with a recovery higher than 70% and a CV < 20%. Concentration values below the quantification limit were not considered for the kinetic analysis of experimental data. The linear regression lines for IVM and MXD showed correlation coefficients ⩾ to 0.999. The mean recoveries of IVM and MXD from plasma were in a range between 72 and 92%. The inter assay precision of the analytical procedures obtained after HPLC analysis of IVM on different working days showed CV < 10%. The limit of quantification was established at 0.2 ng/ml.

4. Results

The FECRT values confirmed the presence of resistant nematode populations. The efficacy estimations for the different treatments based on both the WAAVP and Abbott formulae did not show significant differences. The reduction percentages of nematode faecal egg counts were 23% (IVM) and 69% (MXD). Mean pre-treatment percentages of larvae recovered from cultures showed that *Cooperia* spp. (62.2%) and *Ostertagia* spp. (32.8%) were the predominant nematodes present. Lower percentages of *Haemonchus* spp. (3.5%), *Oesophagostomum* spp. (0.5%) and *Trichostrongylus* spp. (1.0%) larvae were recovered. After treatment with IVM and MXD, *Cooperia* spp. (between 92.9 and 99.1%) was the predominant nematode genera. The presence of LPM tended to enhance the efficacy of both ML compounds. The FECRT values rose up to 50 (IVM) and 87% (MXD) (WAAVP method) after the co-administration with LPM. The animals treated with both ML plus LPM had significantly lower ($P < 0.05$) epg counts compared to that obtained in the untreated control group. Table 1 summarizes the anthelmintic efficacy obtained after the different drug treatments. The third stage (L3) larvae composition (%) observed after faecal culture of the pooled faecal samples is shown in Table 2. The comparative mean number of eggs per gram of faeces obtained in animals from the untreated (control) and IVM/MXD treated groups (with and without LPM), is shown in Fig. 1.

Both IVM and MXD parent compounds were recovered in plasma up to 30 days post-treatment. MXD showed a faster absorption from the administration site and extended persistence in the bloodstream compared to IVM. The times to peak plasma concentration were 0.47 (MXD) and 2.37 days (IVM). The slow elimination of MXD from the body was reflected by the elimination half-life values. Whereas MXD elimination half-life was 13.3 days, the mean value obtained for IVM was 5.50 days. The presence of LPM modified the plasma disposition of both IVM and MXD. The plasma concentration profiles for IVM and MXD were higher after their co-administration with LPM in comparison to those observed after their administration alone. The IVM and MXD plasma concentration profiles obtained after their administrations either alone and co-administered with LPM in cattle are compared in Fig. 2 and 3. The higher IVM and MXD plasma profiles observed in the presence of LPM accounted for an enhanced systemic availability of both ML compounds. The total area under the concentration vs. time curves (AUC) for IVM and MXD were between 20 and 54% greater after their co-administration with the P-glycoprotein modulator. The comparative pharmacokinetic parameters obtained after the administration of IVM and MXD either alone or co-administered with LPM in cattle are shown in Table 3.

5. Discussion

The understanding of the relationship between pharmacokinetics and pharmacodynamics (efficacy) of anthelmintic drugs is crucial to maximize the antiparasitic control in livestock. This concept has recently acquired a great relevance when resistance to different families of anthelmintic compounds has become widespread (Kaplan, 2004). The IVM and MXD pharmacokinetic patterns described here in cattle agree with the available information previously described in this species (Lifschitz et al., 1999a,b, 2000). These ML compounds are highly lipophilic substances with an extensive distribution to tissues, including sites of parasite location (Lifschitz et al., 1999a,b, 2000). Their long persistence after the subcutaneous administration to cattle is based on the deposit of active drug in fatty tissues (Lanusse et al., 1997). However, the elevated lipophilicity of MXD (100 times higher than IVM) (Hennessy, 1997) results in a greater affinity of this molecule for fat compared to that of IVM. The higher lipophilicity and the lower affinity by the P-gp for MXD compared to IVM (Lespine et al., 2007) may contribute to the different persistence of both drugs in the body. Accordingly, the plasma mean residence time for MXD was significantly longer (17 days) compared to that obtained for IVM (8.43 days) in the current trial.

Table 1
Results of the faecal egg counts reduction test (FECRT) obtained after ivermectin (IVM) and moxidectin (MXD) subcutaneous administration either alone or co-administered with loperamide (LPM) (SC, 0.4 mg/kg) to cattle.

Treatment group	IVM alone	IVM + LPM	MXD alone	MXD + LPM
FECRT (%)[a] (WAAVP)	23.5	50.0	69.5	87.1
FECRT (%)[b] (Abbott)	37.5	62.1	74.0	79.0

[a] Calculated following Coles et al. (1992).
[b] Calculated following Dash et al. (1988).

Table 2
Third stage (L3) larvae composition (%) observed after faecal culture of pooled faecal samples obtained from untreated and treated calves at 14 days post-treatment.

Treatment group	*Ostertagia* spp.	*Trichostrongylus* spp.	*Cooperia* spp.	*Haemonchus* spp.	*Oesophagostomum* spp.
Untreated control	32.8	1.0	62.2	3.5	0.5
IVM	0.0	1.6	98.4	0.0	0.0
IVM + LPM	4.4	0.0	95.6	0.0	0.0
MXD	0.0	0.9	99.1	0.0	0.0
MXD + LPM	7.1	0.0	92.9	0.0	0.0

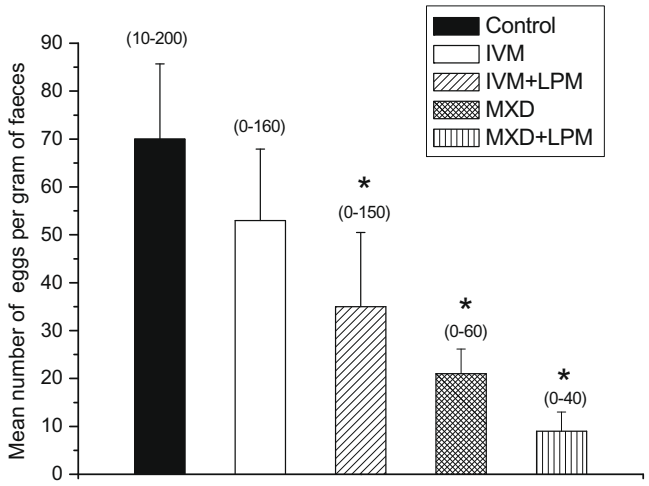

Fig. 1. Mean eggs per gram of faeces (epg) counts in the untreated (control), IVM and MXD treated animals obtained after 14 days of their subcutaneous administration either alone or co-administered with loperamide (LPM) (SC, 0.4 mg/kg) to cattle. The range of the egg counts is shown in brackets for each experimental group. (*) Mean values are significantly different from those obtained for control group at $P < 0.05$.

The faecal egg count reduction values observed in the current trial are consistent with a high level of resistant of the nematode population under study to both anthelmintic drugs. The percentages of egg counts reduction obtained after the utilization of both the WAAVP and Abbott formulae were similar and are in agreement with a report by Suarez and Cristel (2007) working with cattle from the same geographical area. The predominant nematode found after the IVM and MXD treatments was *Cooperia* spp., as was corroborated in a previous survey performed in the same region (Suarez and Cristel, 2007). Moreover, *C. oncophora* highly resistant to IVM was recovered from sacrificed treated calves from the same farm (Suarez et al., unpublished data).

The reduction percentage value obtained after the MXD treatment was 69% compared to 23% observed after IVM administration. The mean number of eggs per gram of faeces was not significantly different after IVM treatment compared to the control group (Fig. 3). Earlier publications have shown a higher efficacy for MXD than for IVM against resistant nematodes in sheep (Craig et al., 1992; Le Jambre et al., 1995). The antiparasitic activity of IVM and MXD has been proposed to be due to their effects on irreversibly opening glutamate-gated chloride channels (Shoop et al., 1995). Channels from susceptible and IVM-resistant *C. oncophora* were significantly more sensitive to MXD than to IVM (Njue et al., 2004). The higher drug potency (Njue et al., 2004) and the lower affinity by P-gp (Lespine et al., 2007) of MXD compared to IVM may explain the higher efficacy obtained against resistant *Cooperia* spp. in the current trial.

The implications of different cell transporter systems in the processes of drug absorption, distribution and metabolism/excretion have been studied in the last few years. Among all the identified cell transporters, P-gp has been the most studied (Lin, 2003). In the mammalian host, P-gp participates in the mechanism of active biliary and intestinal secretion of different molecules from the bloodstream to the gastrointestinal tract (Lin, 2003). Abamectin, IVM and MXD (Lespine et al., 2007) have been shown to be inhib-

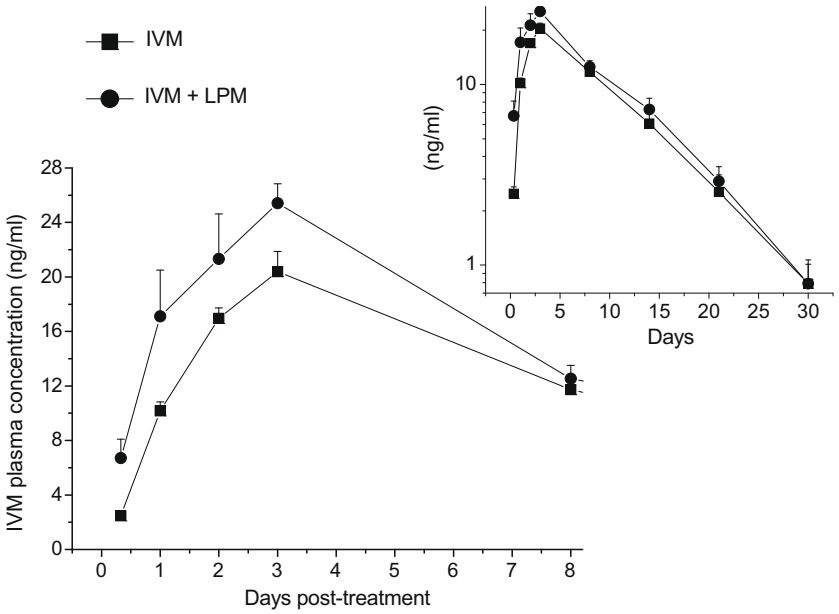

Fig. 2. Mean (±SD) ($n = 6$) ivermectin (IVM) plasma concentrations measured after its subcutaneous administration either alone at recommended dose or co-administered with loperamide (LPM) (SC, 0.4 mg/kg) to cattle infected with resistant nematodes.

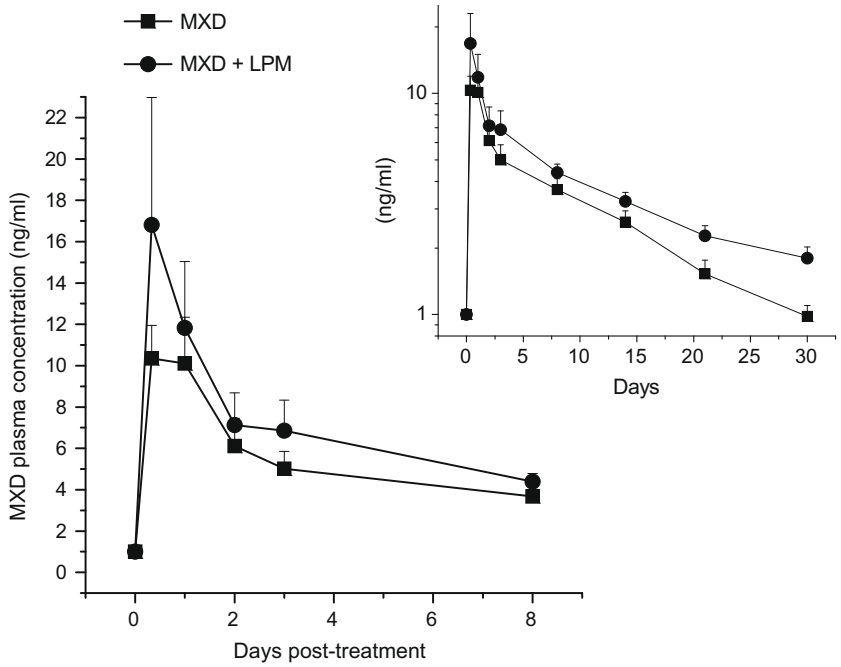

Fig. 3. Mean (±SD) ($n = 6$) moxidectin (MXD) plasma concentrations measured after its subcutaneous administration at recommended dose either alone or co-administered with loperamide (LPM) (SC, 0.4 mg/kg) to cattle infected with resistant nematodes.

Table 3
Mean (±SD) ($n = 6$) plasma kinetic parameters obtained for ivermectin (IVM) and moxidectin (MXD) after its subcutaneous administration either alone or co-administered with loperamide (LPM) (SC, 0.4 mg/kg) to cattle.

Kinetic parameters	IVM alone	IVM + LPM	MXD alone	MXD + LPM
T_{max} (days)	2.67 ± 0.40	2.83 ± 0.40	0.44 ± 0.30	0.55 ± 0.30
C_{max} (ng/ml)	20.4 ± 3.18	26.4 ± 4.20*	11.5 ± 5.19	17.3 ± 14.6
$AUC_{0-8\ d}$ (ng d/ml)	116 ± 13.8	149 ± 23.1*	43.5 ± 14.2	57.7 ± 28.8
AUC_{total} (ng d/ml)	215 ± 37.8	259 ± 33.7*	108 ± 23.8	166 ± 35.0*
MRT (days)	8.32 ± 1.40	8.05 ± 1.28	17.0 ± 4.56	24. 5 ± 12.4
$T½$ el (days)	5.50 ± 1.65	5.30 ± 1.26	13.3 ± 4.07	16.4 ± 8.30
Cl_B/F (l/kg d)	0.93 ± 0.16	0.77 ± 0.10*	1.94 ± 0.54	1.24 ± 0.21*

T_{max}: time to peak plasma concentration. C_{max}: peak plasma concentration. AUC_{total}: area under the concentration vs time curve extrapolated to infinity. MRT: mean residence time. $T½$ el: elimination half-life. Cl_B/F: total body clearance, which represents its true value divided by the bioavailability (F).

* Mean kinetic parameters are significantly different from those obtained for the IVM and MXD alone treatments at $P < 0.05$.

itors of the P-gp transport protein. Different approaches have been followed to evaluate the relevance of the intestinal P-gp mediated secretion of ML. The transepithelial intestinal secretion plays a major role in the elimination of ivermectin in the rat (Laffont et al., 2002). Recent *in vitro* work, performed using the everted sac technique, demonstrated a marked increase on the IVM accumulation rate in the ileum wall in the presence of the P-gp modulator itraconazole (Ballent et al., 2006). In this context, changes to the ML plasma disposition are expected when these compounds are co-administered with P-gp modulators. Changes to the pharmacokinetic behavior of IVM and MXD after their co-administration with the opioid derivative LPM have been observed in different animal species. Significantly higher MXD plasma concentrations and delayed faecal elimination were observed after MXD + LPM administration compared to MXD alone in cattle (Lifschitz et al., 2002). The co-administration of IVM + LPM in rats led to higher IVM concentrations in plasma, liver and small intestine mucosa (Lifschitz et al., 2004). The outcome of the current work demonstrates that LPM co-administration modified the plasma disposition of both IVM and MXD in cattle. The availability of both active ingredients in the bloodstream (measured as AUC values) increased from 215 to 259 ng d/ml (IVM) and from 108 to 166 ng d/ml (MXD), which correlates with a significantly decreased body clearance observed for both molecules in calves receiving the co-administration with LPM.

It is interesting to notice that the ML molecules appear to have different P-gp efflux potential, with an IC_{50} for IVM approximately 10 times lower than that reported for MXD (Lespine et al., 2007). In agreement, IVM but not MXD availability was increased after their co-administration with the P-gp modulating agent verapamil in sheep (Molento et al., 2004). However, LPM induced changes to the pharmacokinetic behavior of both ML in the present study in cattle. The altered IVM and MXD disposition kinetics induced by LPM may be based on a P-gp interaction affecting the active intestinal secretion of both molecules. Additionally, decreased biliary secretion and delayed gastrointestinal transit time may also contribute to explain the observed changes to the disposition of IVM and MXD.

P-gp has also been identified in parasites such as *Onchocerca volvulus* (Kwa et al., 1998) and *Haemonchus contortus*, which contains at least four P-gp genes (Sangster and Gill, 1999). Data supporting the association between P-gp and resistance to IVM are now available. A number of different P-gps are overexpressed in resistant *H. contortus* (Prichard and Roulet, 2005). It was reported that IVM and MXD treatments select for a constitutive or inducible over-expression of at least five P-gps in adult *H. contortus* (Prichard and Roulet, 2007). The use of the multiple drug resistant (MDR)-reversing agents, verapamil and CL347099, increased the efficacy of IVM and MXD against resistant *H. contortus* strains in jirds (Molento and Prichard, 1999). Considering this background information, the *in vivo* modulation of the P-gp-mediated drug efflux may be a useful pharmacological strategy to increase both the drug systemic availability and the efficacy against resistant nematodes in farm animals.

The presence of LPM suggested a modification in the efficacy against resistant parasites. The egg output reduction values increased from 23% to 50% (ivermectin) and from 69% to 87% (moxidectin) following their co-administration with LPM. As above

stated, the reduced affinity by P-gp previously demonstrated for MXD compared to IVM, may explain the differential increase observed in efficacy after their co-administration with LPM. While the presence of LPM tended to enhance MXD anthelmintic efficacy (estimated by the FECRT) to change from 1.05- (Abbot) and 1.28-fold (WAAVP), IVM activity changed from 1.66- (Abbot) and 2.12-fold (WAAVP) in the presence of LPM.

The enhanced IVM and MXD systemic concentration profiles obtained in co-administered calves and also the reduction on P-gp drug efflux induced by LPM at the target nematode, may together account for the higher active concentrations at the receptor site of action. Alternative mechanisms may also help to increase the efficacy after the modulation of P-gp. Cell apoptosis was described after exposing cells to some P-gp inhibitors (Karwatsky et al., 2003). If such an apoptotic mechanism occurs in target nematodes (see Lespine et al., 2008), the presence of LPM as a P-gp modulating agent may act complementary to the enhanced drug concentrations to obtain an advantageous antiparasitic action. The complex molecular mechanisms involved in this type of drug–drug interaction require further evaluations at the host and parasite levels. Although avermectins such as IVM have been shown to have high affinity by P-gp, it has been also reported that IVM may interact with other cell transport proteins such as multidrug resistance proteins (MRP1, MRP2 and MRP3) (Lespine et al., 2006, 2007), while MXD has affinity by the breast cancer resistant protein (BCRP) (Perez et al., 2009).

The pharmaco-parasitological impact of P-gp modulation in cattle naturally infected with resistant ML nematodes is reported for the first time. The co-administration of ML compounds with a P-gp modulator induced pharmacokinetic modifications, which produced a tendency towards enhanced efficacy against *Cooperia* spp. highly resistant to both antiparasitic compounds. Potential side effects of the modulating agents, their relatively short persistence and changes to the pattern of tissue residues (Lespine et al., 2008) are among issues to be addressed before any practical applications can be advised. However, the work reported here contributes with further evidence on the high degree of resistance to ML in cattle nematodes and, shows under field conditions, that modulation of P-gp may be a valid pharmacological approach to improve the activity and extend the lifespan of these antiparasitic molecules in veterinary medicine.

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