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Interference with P-glycoprotein improves ivermectin activity against adult resistant nematodes in sheep

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ABSTRACT

The *in vivo* co-administration of ivermectin (IVM) with P-glycoprotein (P-gp) modulator agents has been shown to enhance its systemic availability. However, there is no sufficient evidence on the impact that this type of drug–drug interaction may have on the *in vivo* efficacy against resistant nematodes in ruminant species. The current work reports on the effects of loperamide (LPM), a P-gp modulating agent, on both IVM kinetic behaviour and anthelmintic activity in infected lambs. Eighteen (18) lambs naturally infected with IVM-resistant gastrointestinal nematodes were allocated into three (3) experimental groups. Group A remained as untreated control. Animals in Groups B and C received IVM (200 μg/kg, subcutaneously) either alone or co-administered with LPM (0.2 mg/kg, twice every 12 h), respectively. Individual faecal samples were collected from experimental animals at days −1 and 14 post-treatment to perform the faecal eggs count reduction test (FECRT). Blood samples were collected between 0 and 14 days post-treatment and IVM plasma concentrations were determined by HPLC. Additionally, at day 14 post-treatment, lambs from all experimental groups were sacrificed and adult gastrointestinal nematode counts were performed. FECRT values increased from 78.6 (IVM alone) to 96% (IVM + LPM). *Haemonchus contortus* was highly resistant to IVM. The IVM alone treatment was completely ineffective (0% efficacy) against adult *H. contortus*. This efficacy value increased up to 72.5% in the presence of LPM. The efficacy against *Trichostrongylus colubriformis* increased from 77.9% (IVM alone) to 96.3% (IVM + LPM). The described favorable tendency towards improved anthelmintic efficacy was in agreement with the enhanced IVM plasma availability ($P < 0.05$) and prolonged elimination half-life ($P < 0.05$) induced by LPM in infected lambs. A LPM-induced P-gp modulation increases IVM systemic exposure in the host but also it may reduce P-gp efflux transport over-expressed in target resistant nematodes.

1. Introduction

Ivermectin (IVM) is the most used anthelmintic drug for parasite control in livestock animals. Since its introduction into the veterinary market in 1981, IVM has been extensively used against endo- and ectoparasites rapidly becoming in a leading drug compound worldwide (Crump and Otoguro, 2005). After several years of intensive use to optimize animal productivity, the inevitable appearance of

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IVM-resistant parasites has occurred (Geary, 2005). Currently, resistance to IVM and to other related macrocyclic lactones (MLs), is widespread in nematodes from small ruminants and it is becoming a serious concern in cattle nematodes (Kaplan, 2004; Anziani et al., 2004; Demeler et al., 2009a).

The ATP-binding cassette (ABC) transporters, such as P-glycoprotein (P-gp), have been implicated on the mechanisms of resistance in nematodes (Xu et al., 1998; Prichard and Roulet, 2007). P-gp is a transmembrane protein associated with a phenotype of multidrug resistance 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 (Ling and Thompson, 1974). IVM behaves as a P-gp substrate (Schinkel et al., 1994) and a drug efflux P-gp-mediated process reducing IVM concentrations at the parasite site of action was proposed as one of the resistance mechanisms in *Haemonchus contortus* (Blackhall et al., 1998; Prichard and Roulet, 2007).

Besides its contribution to resistance mechanisms, P-gp activity has also been observed in healthy tissues, particularly in organs relevant to drug disposition kinetics. According to its specific location at the apical side of epithelial cells, luminal surface of hepatocytes and ducts cells, kidney proximal cells and enterocytes, P-gp plays an important role in how drug compounds are absorbed, distributed to tissues and excreted from the body (Lin, 2003). The *in vivo* co-administration of MLs with P-gp modulator agents has been evaluated in different species (Lifschitz et al., 2002, 2004; Dupuy et al., 2003; Ballent et al., 2007; Alvinerie et al., 2008). Loperamide (LPM) is an opioid derivative that has been classified as a P-gp substrate (Schinkel et al., 1996). LPM induces favorable changes to the plasma kinetic disposition of moxidectin and IVM without any toxic effect (Lifschitz et al., 2002, 2004). However, there is not sufficient evidence on the impact that this type of drug–drug interaction may have on the *in vivo* field efficacy against resistant nematodes in sheep. It has been recently shown that modulation of P-gp activity improves the faecal egg count reduction induced by both IVM and moxidectin in cattle infected with resistant nematodes (Lifschitz et al., 2010). The current work describes for the first time a simultaneous pharmaco-parasitological assessment of the effects of LPM, a P-gp modulating agent, on IVM anthelmintic activity in sheep. LPM-induced changes on both IVM plasma disposition and efficacy (controlled test) were assessed in lambs naturally infected with highly resistant gastrointestinal nematodes.

2. Materials and methods

2.1. Animals

Eighteen (18) Corriedale lambs (26–35 kg), naturally infected with resistant GI nematodes were involved in this trial. The selected farm is a sheep experimental unit with a parasite control program based on the intensive use of anthelmintics over the years, where failure of IVM to control nematodes was previously corroborated (Entrocasso, C., personal communication). In fact, a 79.8% reduction on faecal egg counts was obtained after treatment with IVM the year prior to performing the trial described here (Entrocasso et al., 2008). The selection of the animals was based on worm egg per gram counts (epg). On day −1 all lambs were checked for epg, ear tagged and the individual body weights were recorded. Experimental animals had an average of 4538 ± 1370 epg counts ranging from 2520 to 6960. Animals were allocated in a paddock and feed on a lucerne/white and red clover pasture during the experiment and for 20 days before start clinical efficacy study. All the animals had free access to water. Animal procedures and management protocols were approved by the Ethics Committee according to the Animal Welfare Policy (act 087/02) of the Faculty of Veterinary Medicine, Universidad Nacional del Centro de la Provincia de Buenos Aires (UNCPBA), Tandil, Argentina (http://www.vet.unicen.edu.ar).

2.2. Experimental design, treatments and samplings

Experimental lambs were assigned into three (3) experimental groups. Group A remained as untreated control. Animals in Groups B and C received IVM (Ivomec®, Merial Argentina) (200 μg/kg, subcutaneuosly) either alone or co-administered with LPM (0.2 mg/kg, two subcutaneous injections administered at 0 and 12 h post-administration of IVM) (Loperol, Argos, Argentina). LPM was administered at a therapeutic dose rate, and no adverse side effects were observed. Faecal samples were collected from all the lambs in each experimental group at days −1 and 14 post-treatment in order to estimate the epg counts. Jugular blood samples (7 ml) were collected into heparinised vacutainer tubes prior to and at 3, 6, 9 h and 1–4, 6, 8, 10 and 14 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).

Additionally, at 14 days post-treatment, all the animals in each experimental group were sacrificed by captive bolt gun and rapidly exsanguinated. Abomasum and different gut sections were identified and isolated (small and large intestine) and the content analyzed to record the different parasite stages following the World Association for the Advancement of Veterinary Parasitology guidelines (Wood et al., 1995).

3. Analytical procedures

3.1. Parasitological techniques

The individual faecal egg counts were performed using the modified McMaster technique (Roberts and O'Sullivan, 1949). The anthelmintic efficacy of the treatments was evaluated by the faecal egg count reduction test (FECRT), calculated according to the formula (Coles et al., 1992):

$$\text{FECRT}(\%) = 100 \times \left(1 - \frac{T}{C}\right),$$

where T is the arithmetic mean epg counts in the treated group at 14 days post-treatment and C is the arithmetic

mean epg counts in the untreated control group at 14 days post-treatment. The 95% confidence intervals were calculated as reported by Coles et al. (1992). Direct adult nematode counts of animals from experimental groups were determined 14 days after treatment according to the World Association for the Advancement of Veterinary Parasitology (WAAVP) guidelines (Wood et al., 1995). The efficacy of each anthelmintic treatment was determined by the comparison of worm burdens in treated versus untreated animals. The following equation expresses the percentage of efficacy (%E) of a drug treatment against a given parasite species (S) in a single treatment group (T) when compared with an untreated control (C):

$$\%E = \left[\frac{\text{Mean of S in C} - \text{Mean of S in T}}{\text{Mean of S in C}}\right] \times 100$$

The geometric mean was used according with recommendation of Wood et al. (1995).

3.2. Pharmacological determinations

3.2.1. IVM chemical extraction and derivatization

The extraction of IVM from spiked and experimental plasma samples was carried out following the technique first described by Alvinerie et al. (1993), slightly modified by Lifschitz et al. (1999). 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 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 were detected with a fluorescence detector (Shimadzu, RF-10 Spectrofluorometric detector, Kyoto, Japan), reading at 365 nm (excitation) and 475 nm (emission wavelength). IVM 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 was performed before starting analysis of the experimental samples obtained during the pharmacokinetic trial. Calibration curves in the range between 0.2 and 50 ng/ml were prepared. 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 recovery from plasma were obtained in the range between 0.2 and 50 ng/ml. The precision of the extraction and chromatography procedures was estimated by processing replicate aliquots (n = 4) of pooled lambs plasma samples containing known IVM 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 showed correlation coefficients ≥0.99. The mean recoveries of IVM from plasma were in a range between 75 and 82%. 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 0.1 ng/ml.

4. Pharmacokinetic analysis of the data

The plasma concentrations versus 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 non-compartmental model method. The peak concentration (C_{max}) was read from the plotted concentration–time curve in each individual animal. The area under the concentration vs. time curves (AUC) were calculated by the trapezoidal rule (Gibaldi and Perrier, 1982) and further extrapolated to infinity by dividing the last experimental concentration by the terminal slope (λz). The terminal (elimination) half-life ($t_{1/2el}$) was calculated as $\ln 2/\lambda z$. IVM plasma concentrations and all the estimated pharmacokinetic parameters are reported as mean ± S.D.

5. Statistical analysis

Faecal egg and nematode counts (reported as arithmetic mean ± S.D.) were analyzed by non-parametric ANOVA (Kruskal–Wallis test). Mean pharmacokinetic parameters for IVM were statistically compared using Student's t-test. The assumption that the data obtained after treatments have the same variance was assessed. A non-parametric Mann–Whitney test was used where significant differences among standard deviations were observed. The statistical analysis was performed using the Instat 3.0 Software

Table 1
Nematode egg counts* (range) and reduction percentage of faecal egg counts (FECRT) in the untreated (control) and ivermectin (IVM) treated animals (0.2 mg/kg) obtained after their subcutaneous administration either alone or co-administered with loperamide (LPM) (0.2 mg/kg, twice every 12 h) to lambs.

Treatment group	Mean epg* (range)		FECRT (%)	UCL	LCL
	Day −1	Day 14			
Untreated group	4085 (2520–5760)	28136[a] (3360–90360)	–	–	–
IVM alone	5045 (3510–6960)	6010[a] (640–11400)	78.6	94	23.6
IVM + LPM	4485 (2640–6960)	1126[b] (60–2700)	96	99	83.7

* Arithmetic mean of eggs per gram of faeces; UCL: upper confidence limit 95%; LCL: lower confidence limit 95%. Nematode egg counts at day 14 post-treatment with different superscripts are statistically different at $P < 0.05$.

(Graph Pad Software, CA, USA). A value of $P < 0.05$ was considered statistically significant.

6. Results

The faecal egg counts obtained for all experimental groups, including the results of the FECRT and upper and lower confidence limits (95%) are shown in Table 1. The low percentage of reduction in the eggs number in faeces and the limited efficacy results obtained in the controlled test indicate the presence of gastrointestinal (GI) nematodes resistant to IVM. The FECRT reached a 78.6% after the IVM alone treatment. The egg counts in the animals treated with IVM alone were not significantly different compared to those obtained in the untreated control at day 14 post-administration. *H. contortus* showed to be highly resistant to IVM with a mean worm burden reduction of 0% compared to the control group.

The efficacy of IVM against intestinal nematode parasites such as *Trichostrongylus colubriformis* and *Nematodirus* spp. was below 90%. Adult nematode counts were not statistically different between IVM-treated and untreated control lambs, indicating a reduced susceptibility of these parasite species.

The co-administration with LPM increased IVM nematodicidal efficacy. The FECRT reached a value of 96% and the epg counts in lambs treated with IVM + LPM were significantly lower ($P < 0.05$) compared to those obtained in both the untreated control and IVM alone treated animals. The efficacy against the resistant nematodes enhanced in the presence of LPM with a percentage of reduction of 72.5% (*H. contortus*), 96.3% (*T. colubriformis)* and 93.0% (*Nematodirus* spp.). The adult nematode counts and resultant clinical efficacy obtained for the IVM alone and IVM + LPM treatments are shown in Table 2.

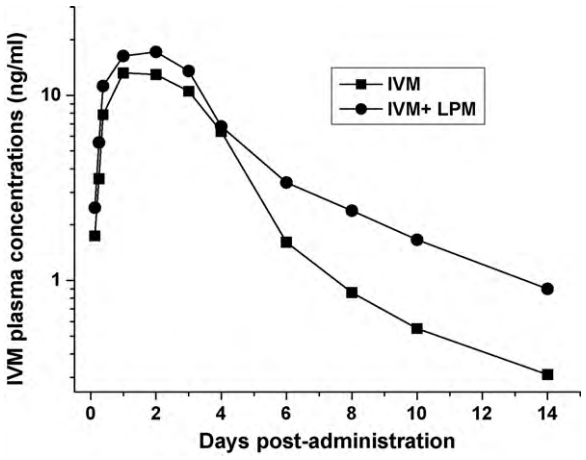

Fig. 1. Plasma concentration profiles of ivermectin (IVM) ($n = 6$) obtained after its subcutaneous administration either alone (0.2 mg/kg) or co-administered with loperamide (LPM) to lambs infected with resistant nematodes.

IVM parent compound was recovered in plasma up to 14 days post-treatment in both treated groups. Higher IVM concentrations were obtained after its co-administration with LPM in comparison to those observed after its administration alone. The IVM plasma concentration profiles obtained after its administration either alone and co-

Table 2
Nematode worm counts (geometric mean and range) and efficacy obtained after 14 days post-treatment with ivermectin (IVM) (0.2 mg/kg) subcutaneously administered either alone or co-administered with loperamide (LPM) (0.2 mg/kg, twice every 12 h) to lambs. Nematode worm count recorded in the untreated control group is also shown.

Parasites	Untreated group	IVM alone		IVM + LPM	
	Worm counts	Worm counts	Efficacy (%)	Worm counts	Efficacy (%)
Abomasum					
Haemonchus spp.	276[a] (100–1600)	488[a] (300–900)	0	76[a] (0–1000)	72.5
Teladorsagia spp.	632[a] (200–1500)	0[b]	100	0[b]	100
Trichostrongylus axei	485[a] (200–800)	6.83[b] (0–1000)	98.6	1.82[b] (0–500)	99.6
Small intestine					
Trichostrongylus colubriformis	4705[a] (1900–10400)	1040[ab] (100–9300)	77.9	174[bc] (0–3400)	96.3
Nematodirus spp.	484[a] (200–1100)	70[ab] (0–1900)	85.5	34[bc] (0–900)	93.0
Large intestine					
Oesophagostomun spp.	10[a] (0–200)	0[b]	100	0[b]	100

Adult nematode counts at day 14 post-treatment with different superscripts are statistically different at $P < 0.05$.

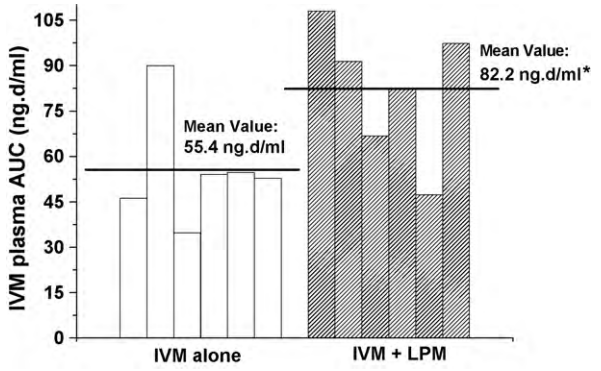

Fig. 2. Individual values of area under the concentration vs. time curve (AUC) for ivermectin (IVM) obtained after its subcutaneous administration either alone (0.2 mg/kg) or co-administered with loperamide (LPM) to lambs infected with resistant nematodes. (*) Values are significantly different from those obtained for IVM alone treatment at $P < 0.05$.

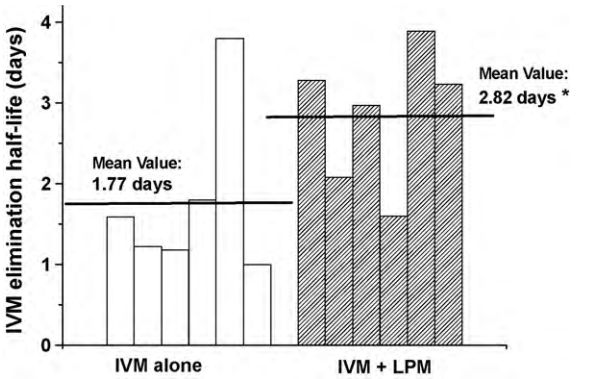

Fig. 3. Individual values of elimination half-life ($t_{1/2el}$) ($n = 6$) for ivermectin (IVM) obtained after its subcutaneous administration either alone (0.2 mg/kg) or co-administered with loperamide (LPM) to lambs infected with resistant nematodes. (*) Values are significantly different from those obtained for IVM alone treatment at $P < 0.05$.

administered with LPM in infected lambs are compared in Fig. 1.

The higher IVM plasma profiles observed in the presence of LPM accounted for an enhanced systemic availability. The mean AUC value for IVM increased from 55 (IVM alone) to 82 ng d/ml (IVM + LPM). The IVM elimination half-life was prolonged in the presence of the P-gp modulator (1.60-fold). The comparative pharmacokinetic parameters obtained after the administration of IVM either alone or co-administered with LPM are shown in Figs. 2 and 3, respectively. The enhancement in the IVM plasma AUC obtained after its co-administration with LPM accounted for reduced epg counts and lower adult nematode counts. There was an inverse correlation between drug systemic availability (AUC values) and total adult nematode counts (r: 0.65, p: 0.02). The relationship among systemic plasma exposure (AUC), epg and adult nematode counts are shown in Fig. 4.

7. Discussion

After 28 years of intensive use with a high selection pressure on parasite population, the development of resistance to IVM is a seriously increasing problem in ruminants (Kaplan, 2004; Wolstenholme et al., 2004). The IVM pharmacokinetic patterns described here in lambs agree with the available information previously described in the ovine species (Barber et al., 2003; Imperiale et al., 2004; El-Banna et al., 2008). However, the IVM concentrations measured in plasma in the current trial after its subcutaneous administration were not sufficient to achieve an optimal efficacy against different GI nematode parasites. According to the

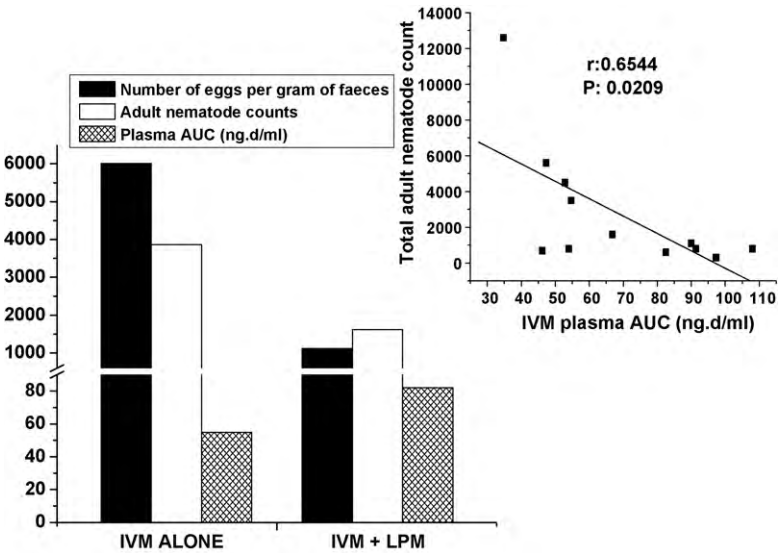

Fig. 4. Relationship between the number of eggs per gram in faeces, the total adult nematode counts and the systemic availability (area under the plasma concentration vs time curve) (AUC), obtained after ivermectin (IVM) subcutaneous administration either alone (0.2 mg/kg) or co-administered with loperamide (LPM) to lambs infected with resistant nematodes. The insert shows the inverse correlation between adult nematode counts and the IVM plasma AUC values. r: coefficient of correlation. Value statistically significant at $P < 0.05$.

criteria from Coles et al. (1992) in evaluating anthelmintic resistance by means of the FECRT, it is clear that resistance against IVM was present in the nematode population involved in the described trial. The FERCT for IVM reached a 78.6 with a 95% confidence interval ranging from 23.6 to 94 (Table 1). The reduced IVM activity against some nematode species was confirmed in the controlled efficacy test. *H. contortus* showed to be highly resistant to IVM (completely ineffective with 0% efficacy). On the other hand, the efficacy against other abomasal parasites such as *Teladorsagia* spp. and *Trichostrongylus axei* was optimal (Table 2). In small intestine, a reduced IVM efficacy (below 90%) was observed against *T. colubriformis* (77.9%) and *Nematodirus* spp. (85.5%) with nematode worm counts resulting not statistically different from those obtained in the untreated control group. Despite the deep knowledge achieved on the understanding of the pharmacological properties of the MLs, the drug concentrations required *in vivo* to inhibit parasite establishment or larval development have not yet been determined. A differential susceptibility to drug activity among different GI nematodes and the complexity for developing *in vitro* culture models for adult worms have accounted for a lack of information on the therapeutically relevant concentrations needed to obtain an optimal efficacy against susceptible and resistant parasite strains.

In this context, different pharmacological strategies have been evaluated to modify the pharmacokinetic behaviour and to improve the clinical efficacy of the MLs. Multidrug resistance transporters (MDR) actively drive out many types of xenobiotics from cells (Lin, 2003), including drugs such as the MLs (Lespine et al., 2007). *In vitro* and *in vivo* studies have demonstrated the role of the P-gp mediated intestinal secretion on IVM elimination (Laffont et al., 2002; Ballent et al., 2006). P-gp modulators such as verapamil (Molento et al., 2004), itraconazole (Ballent et al., 2007) and ketoconazole (Alvinerie et al., 2008) have been used to modify the pharmacokinetic disposition of IVM in sheep. The combined administration of MLs and LPM, the latter used as a P-gp modulator agent, produced modifications to the kinetic disposition of these antiparasitic molecules in different animal species. The co-administration of IVM + LPM in rats accounted for a greater systemic exposure and higher IVM concentrations in gastrointestinal tissues (Lifschitz et al., 2004). Significantly higher IVM and moxidectin plasma concentrations and delayed faecal elimination were observed after their co-administration with LPM in cattle (Lifschitz et al., 2002, 2010). The current work confirmed that LPM co-administration modifies the plasma disposition of IVM in sheep (Figs. 1 and 2). The availability of IVM in the bloodstream (measured as AUC values) increased 41% after the co-administration with LPM, which agrees with the enhancement obtained in previous reports in rats (Lifschitz et al., 2004) and cattle (Lifschitz et al., 2002, 2010).

The higher systemic exposure of IVM obtained after the co-administration with the P-gp modulator accounted for a higher efficacy against resistant GI nematodes. The FECRT was 96% and the efficacy against *H. contortus* and *T. colubriformis* increased up to 72.5 and 96.3%, respectively (Tables 1 and 2) in the presence of LPM. P-gp has been described not only in mammals but also in parasites such as *Onchocerca volvulus* (Kwa et al., 1998) and *H. contortus* (Sangster et al., 1999; Prichard and Roulet, 2007). An enhanced P-gp-mediated drug efflux in target parasites has been proposed as a potential resistance mechanism for different antiparasitic compounds (Xu et al., 1998; Kerboeuf et al., 2002; Alvarez et al., 2007). In fact, the scientific evidence supporting this concept has increased during the last few years. IVM and moxidectin induce overexpression of some P-glycoprotein isoforms in nematodes (Prichard and Roulet, 2007). An altered P-gp gene expression pattern was observed between IVM susceptible and resistant isolates of *Teladorsagia circumcincta* (Dicker et al., 2009). Altogether this molecular genetic information may help to understand the results obtained here against a highly resistant nematode population, which demonstrates that *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.

This theoretical assumption on drug–transporter interaction was assessed using *in vitro* assays and clinical efficacy studies in laboratory animals. The efficacy of both IVM and moxidectin against resistant *H. contortus* strains in jirds was increased in the presence of the P-gp modulators verapamil and CL347099 (Molento and Prichard, 1999). It has recently been shown that the presence of P-gp inhibitors increases the *in vitro* sensitivity to IVM in both IVM-sensitive and resistant larvae of *Teladorsagia circumcincta* and *H. contortus* (Bartley et al., 2009a). The presence of the P-gp modulator pluronic 85 in the larval feeding inhibition test produced an average between 46- and 63-fold enhancement in the sensitivity of larvae to IVM (Bartley et al., 2009a). The larval development test and the larval migration inhibition test were also used to study the effect of P-gp modulators (verapamil) on the sensitivity of susceptible and resistant isolates of *Cooperia* spp. The *in vitro* activity of IVM against *Cooperia* spp. was increased between 10- and 100-fold after the co-incubation with the P-gp modulator (Demeler et al., 2009b).

The data obtained in laboratory studies using *in vitro* tests need to be confirmed with *in vivo* trials performed under field conditions. Despite of the increased sensitivity of resistant larvae to IVM after the co-incubation with pluronic 85, the *in vivo* co-administration of IVM with this P-gp modulator to sheep did not show an improved efficacy against resistant *H. contortus* (Bartley et al., 2009b). A field trial done in Argentina (Lifschitz et al., 2010) showed that the efficacy of both IVM and moxidectin against resistant *Cooperia* spp. in cattle tended to increase after their co-administration with LPM as a P-gp modulator. In the same direction, the trial described here showed an *in vivo* positive impact on anthelmintic efficacy induced by P-gp modulation in lambs naturally infected with resistant nematodes. Therefore, it may be assumed that the *in vivo* P-gp-mediated drug–drug interaction increased IVM systemic exposure in the host. However, modulation of the P-gp-mediated efflux transport over-expressed in target resistant nematodes should not be ruled out. The pharmaco-parasitological events taking place after P-gp modulation in the host and target parasite are summarized in Fig. 5.

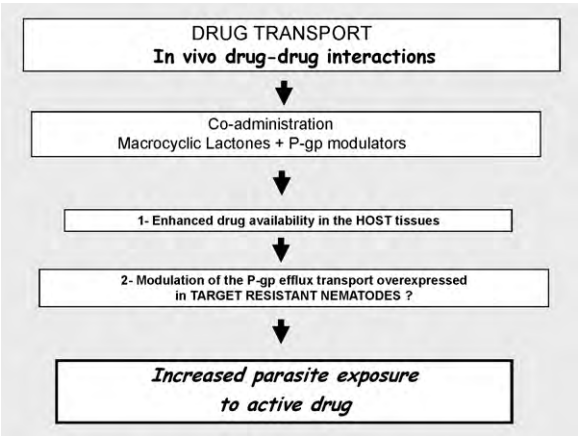

Fig. 5. Schematic representation of the relationship between macrocyclic lactones and P-glycoprotein. The *in vivo* drug–drug interaction between ivermectin and P-glycoprotein modulators accounts for an increased parasite exposure to active drug.

The integration between parasitological and pharmacological information is relevant to improve our understanding of the relationship between drug pharmacokinetics and efficacy. The impact of P-gp modulation in lambs naturally infected with resistant nematodes is reported for the first time. Although further work is required before any practical use is recommended, the results reported here are strong evidence that *in vivo* P-gp modulation may be useful to prolong the effectiveness of some of the antiparasitic compounds at least in the early stages of resistance development.

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