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Loperamide modifies the tissue disposition kinetics of ivermectin in rats

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Abstract

Ivermectin (IVM) is a broad-spectrum antiparasitic drug extensively used in human and veterinary medicine that is largely excreted in bile and faeces. Loperamide (LPM) is an opioid derivative that reduces gastrointestinal secretions and motility. Both IVM and LPM have been reported to act as P-glycoprotein substrates (P-GP). The goal of the present work was to study the LPM-induced modifications to the pattern of tissue distribution for IVM. Thirty-six Wistar male rats were randomly allocated to two groups ($n = 18$) and treated subcutaneously with IVM alone or co-administered with LPM. Rats were killed at different times post-treatment and samples (blood and tissues) were collected and analyzed by HPLC. The presence of LPM induced a marked enhancement in the IVM plasma concentrations, resulting in a significantly higher area under concentration time curve (AUC) value ($P < 0.01$) than that obtained after the administration of IVM alone. Significantly higher IVM availabilities in the liver tissue and small intestine wall ($P < 0.05$) were obtained in the presence of LPM. There were no statistically significant differences in drug availability in the large intestinal wall after both treatments. However, LPM induced a marked decrease in the amount of IVM recovered in the large intestinal lumen content. The ratio between IVM concentrations in the large intestinal luminal content and plasma at day 1 post-treatment was 4.64-fold higher in the absence of LPM. The delayed intestinal transit time caused by LPM accounting for an extended plasma–intestine recycling time, and a potential competition between IVM and LPM for the P-GP-mediated bile–intestinal secretion processes, may account for the enhanced IVM systemic availability reported in the current study.

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Introduction

The macrocyclic lactones (ML) ivermectin (IVM) and moxidectin are potent anthelmintic compounds from the avermectin and milbemycin families, respectively, which are extensively used in veterinary and human medicine worldwide. A single oral dose of $150\,\mu g\,kg^{-1}$ of IVM is extremely effective against the skin microfilariae of *Onchocerca volvulus*, the causative organism of the human onchocerciasis disease known as river blindness (Albiez et al 1996). They are highly potent antiparasitic compounds, known as *endectocide* molecules because of their activity against ecto- and endoparasites (Shoop et al 1995). The high lipophilicity of IVM has been correlated with its extensive distribution to tissues of parasite location and prolonged residence in fat-containing tissues, after its subcutaneous administration to cattle (Lifschitz et al 2000). Large concentrations of unchanged IVM are eliminated by bile and faeces in different animal species (Lifschitz et al 2000; Chiu et al 1990). The ML compounds may be reabsorbed in the intestine after biliary secretion and enterohepatically recycled. It has been shown that up to 24% of doramectin (an avermectin type compound) total dose is secreted in bile in sheep, with 8% of the biliary metabolites being enterohepatically recycled (Hennessy et al 2000). Additionally, it has recently been shown that an active intestinal secretion process is involved in the excretion of unchanged IVM in the rat (Laffont et al 2002).

The widespread use of IVM is important in strategic programs to control parasitism both in domestic animals and humans. However, the appearance of sheep and goat nematodes (Craig & Miller 1990; Echevarria et al 1996; Van Wyk et al 1997) and more

recently cattle nematodes (Fiel et al 2001; Anziani et al 2001) resistant to IVM, and the high selection pressure on *O. volvulus* in humans during the last few years require a better understanding of the pharmacological behaviour of these broad spectrum compounds. It is now well known that the period of time in which an anthelmintic molecule interacts with a target parasite is relevant to achieve optimal antiparasite efficacy (Lanusse & Prichard 1993). Thus, different pharmacological strategies have been investigated to modify the kinetic behaviour of anthelmintic compounds, prolonging their residence time in the bloodstream and consequently in tissues of parasite location (Lanusse et al 1995; Benchaoui & McKellar 1996). Most of the work done in that direction has focussed on interfering with the pattern of drug metabolism of the benzimidazole anthelmintic compounds, such as albendazole and fenbendazole. Alternatively, a drug-induced delay in the pattern of bile–faecal elimination of ML compounds may increase the plasma and target tissue availabilities of the active drug, which will result in enhanced exposure of target parasites to the endectocide molecule.

P-Glycoprotein (P-GP) is a transmembrane protein associated with a phenotype of multidrug resistance (MDR) to certain anticancer drugs (Leveque & Jehl 1995). The role of P-GP in the pharmacokinetic disposition of different drugs has been demonstrated (Schinkel et al 1995; Van Asperen et al 2000). Loperamide (LPM) is an opioid derivative extensively used in veterinary and human medicine whose main pharmacological action is to reduce gastrointestinal secretions and motility. LPM and IVM have been reported as P-GP substrates (Schinkel et al 1994, 1996; Pouliot et al 1997). We have recently demonstrated that LPM induces 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). The objective of the work reported here was to achieve further comprehension of the LPM-induced modifications to the pattern of tissue distribution of ML compounds in an effort to identify a pharmacological approach that could potentiate their antiparasitic activity. An integrated disposition kinetics assessment of the effects of LPM on the concentration profiles of IVM achieved in plasma, liver tissue, small and large intestine tissues and lumen contents in Wistar rats is presented.

Materials and Methods

Experimental animals, treatment and sampling

Thirty-six Wistar male rats weighing 350–400 g were used in the current trial. The management of experimental animals was in agreement with institutional and internationally accepted welfare guidelines (Canadian Council on Animal Care, 1980; American Veterinary Medical Association, 2001). The animals were housed in conventional conditions under controlled temperature and cycles of darkness/light. Rats were randomly allocated into two groups of 18 animals each, with three trial runs in each group. Animals in Group A (IVM alone) received IVM at 200 $\mu g\,kg^{-1}$ (Ivomec, Merial) by subcutaneous injection (SC). The original IVM formulation was diluted in propylene glycol to fit the low volumes of dose. Rats in Group B (IVM + LPM) received IVM SC at the same dose rate co-administered with LPM (propylene glycol:DMSO 90:10 formulation) subcutaneously injected at 1.5 $mg\,kg^{-1}$ in a different injection site. Under slight ether anaesthesia, three animals from each experimental group were killed prior to and at 6, 12, 24, 48 and 72 h post-treatment. Blood was collected by cardiac puncture in heparinised tubes. Samples from liver, small (ileum) and large (caecum) intestine wall tissue and large intestine lumen contents were collected. Blood samples were centrifuged at 2000 *g* for 20 min and the recovered plasma kept in labelled vials. Plasma and tissue samples were rapidly cooled and stored at –20 °C until analysed.

Analytical procedures

IVM concentrations were determined by HPLC with fluorescence detection using automated solid-phase extraction, following a procedure previously described by Alvinerie et al (1993) and Lifschitz et al (2000). Experimental and fortified fluid/tissue samples were homogenised and solid-phase extraction was performed after 15 min incubation at room temperature. A sample aliquot (0.5 g or mL) was combined with 10 ng of internal standard (abamectin) and 0.5 mL of acetonitrile. The mixture was mixed (Multi Tube Vortexer, VWR Scientific Products, USA) for 20 min. After mixing, the tissue samples were sonicated for 10 min (Transsonic 570/H, Lab Line Instruments Inc., USA) and the solvent–sample mixture (plasma or tissues) was centrifuged at 2000 *g* for 15 min. The supernatant was manually transferred to a tube and the procedure repeated once for tissue samples. The pooled supernatants obtained were then placed on the appropriate rack of an Aspec XL autosampler (Gilson, Villiers Le Bell, France). After automated solid-phase extraction, the elute was evaporated to dryness under a gentle stream of dry nitrogen at 60 °C in a water bath. Reconstitution was done using the derivatization method described by De Montigny et al (1990). After completion of the reaction, an aliquot (100 μL) of each sample was injected directly into the chromatograph. The chromatographic conditions included a mobile phase of acetic acid (0.2% in water):methanol:acetonitrile (6:40:54 v/v/v) pumped at a flow rate of 1.5 $mL\,min^{-1}$ through a reverse phase C_{18} column (Selectosil, Phenomenex, Torrance, USA) (5 μm, 4.6 mm × 250 mm). The fluorescence detector (Spectrofluorometric detector RF-10, Shimadzu, Kyoto, Japan) was at an excitation wavelength of 365 nm and an emission wavelength of 475 nm. The extraction recoveries of IVM were > 73% for the different fluids/tissues. The limit of drug detection was established by injection and HPLC analysis of blank plasma fortified with the internal standard and measurement of the baseline noise at the time of retention of the IVM peak. The mean baseline noise plus 10 standard deviations was defined as the theoretical quantification limit. The limit of quantification was

established at 0.2 ng g^{-1} or ng mL^{-1} for the different tissues and fluids. Coefficients of variation $<9\%$ were obtained when the inter-day precision of the chromatographic method was evaluated for the different biological matrices under investigation.

Pharmacokinetic and statistical analysis

The concentration versus time curves obtained for each fluid and tissue analysed were fitted using the PkSolution 2.0 computer program (Ashland, OH). Pharmacokinetic parameters were determined using a non-compartmental method. The peak concentrations (C_{max}) and time to peak concentrations (t_{max}) for each fluid and tissue were read from the plotted concentration–time curves. The area under the concentration–time curves (AUC) was calculated by the trapezoidal rule (Gibaldi & Perrier 1982). Mean pharmacokinetic parameters for IVM obtained after the administration of the IVM alone or co-administered with LPM were statistically compared by Student's *t*-test (Instat 3.0, Graph Pad software Inc., San Diego, CA). The assumption that the data obtained after both treatments have the same variance was evaluated. A log-transformation was used where significant differences among standard deviations were observed. A value of $P < 0.05$ was considered significant.

Results

Ivermectin was recovered from the bloodstream and all the tissues investigated over the 72 h sampling period. A higher IVM plasma concentration profile was obtained after the co-administration of IVM with LPM compared to the treatment with IVM alone. The mean IVM concentrations measured in plasma after both treatments are shown in Figure 1. The presence of LPM induced a marked enhancement in the IVM plasma concentrations, which was between 1.96 and 3.35 fold higher (from 12 to 48 h post-administration) in comparison to IVM administered alone. The higher IVM plasma concentration profiles after the co-administration with LPM resulted in a significantly higher AUC value ($P < 0.01$) than that obtained after the administration of IVM alone.

Marked changes in the IVM tissue concentration profiles were observed as a result of LPM administration. Significantly higher IVM availabilities in the liver tissue and small intestine wall ($P < 0.05$) were obtained in the presence of LPM. At day 1 post-treatment, IVM concentrations in the liver and small intestine wall were between 3- and 4-fold higher than those measured after administration of IVM alone. The IVM concentration profiles measured in the small intestine wall tissue and liver are shown in Figure 2. There were no statistically significant differences in drug availability (expressed as AUC values) in the large intestine wall after both treatments. However, LPM induced a marked decrease in the amount of IVM recovered in the large intestine lumen content. At day 1 post-administration of IVM alone the concentrations of IVM in the large intestine lumen content were 63% higher than those obtained after co-administration with LPM. The IVM concentrations measured in the large intestine wall tissue and lumen content after both treatments are compared in Figure 3. Drug availability in large intestine content was significantly greater after treatment with IVM alone ($P < 0.05$). The ratio large intestine luminal content/plasma at day 1 post-treatment was 4.64-fold higher in the absence of LPM. The ratios between IVM concentrations in the large intestine content and plasma are shown in Figure 4. The pharmacokinetic parameters obtained for IVM after both treatments in all the fluids and tissues are compared in Table 1.

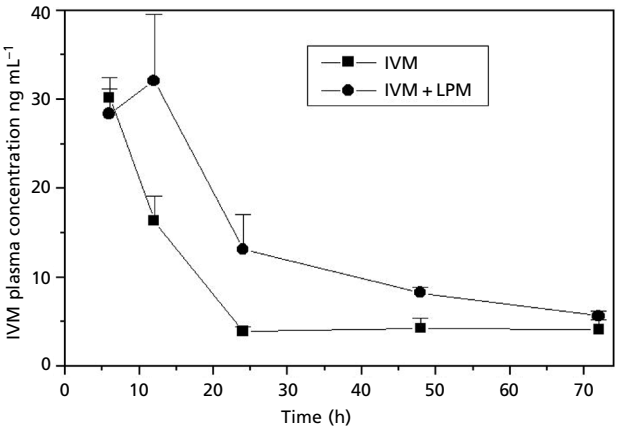

Figure 1 Mean (± s.e.m.) (n = 3 rats per point) IVM plasma concentrations (ng mL^{-1}) obtained after its subcutaneous administration (200 μg kg^{-1}) either alone or co-administered with LPM (subcutaneous at 1.5 mg kg^{-1}) to Wistar rats.

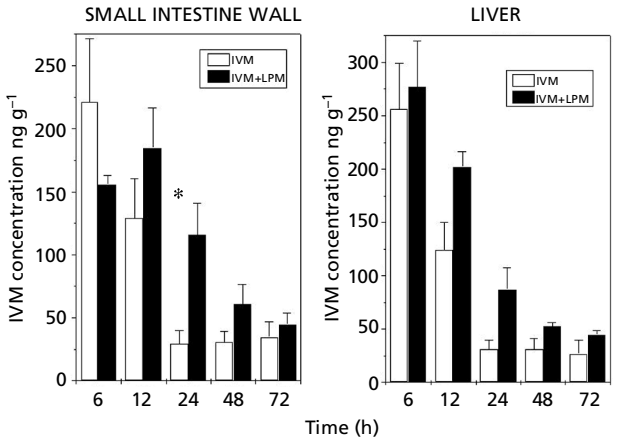

Figure 2 Mean (± s.e.m.) (n = 3 rats per point) IVM concentrations (ng g^{-1}) measured in small intestine wall and liver tissue after its subcutaneous administration (200 μg kg^{-1}) either alone or co-administered with LPM (subcutaneous at 1.5 mg kg^{-1}) to Wistar rats. Values are statistically different from those obtained after IVM alone treatment at $^*P < 0.05$.

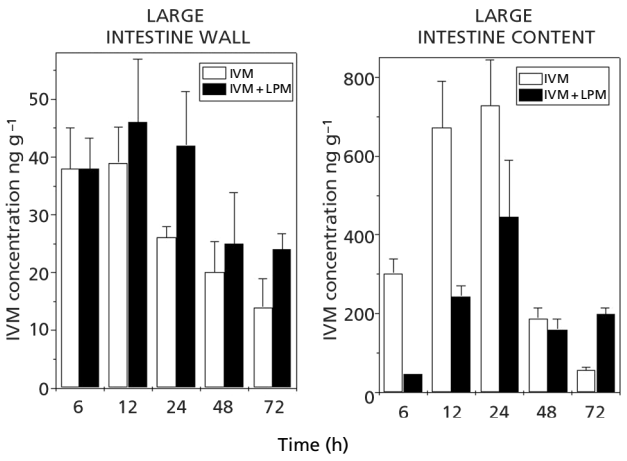

Figure 3 Comparison of IVM concentrations (mean ± s.e.m., n = 3 rats per point) measured in the large intestine tissue wall and its lumen content after subcutaneous administration (200 μg kg^{-1}) either alone or co-administered with LPM (subcutaneous at 1.5 mg kg^{-1}) to Wistar rats. Values are statistically different from those obtained after IVM alone treatment at $^{**}P < 0.01$ and $^{***}P < 0.001$.

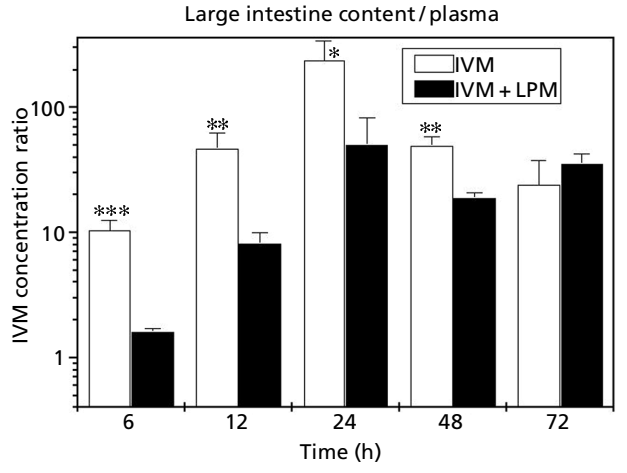

Figure 4 Comparison of the mean ratios between the IVM concentrations recovered in the large intestine lumen content and those measured in plasma after its subcutaneous administration (200 μg kg^{-1}) either alone or co-administered with LPM (subcutaneous at 1.5 mg kg^{-1}) to Wistar rats. Values are statistically different from those obtained after IVM + LPM treatment at $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$.

Discussion

Concomitant administration of different drugs, as is often done in current human medicine and veterinary clinical practice, may drastically modify the systemic availability, disposition kinetics and resultant pharmacological effect of different molecules used in drug therapy. Considering that IVM and LPM are compounds extensively used in human and animal therapeutics, the experiments reported in the present study provide useful pharmacological information regarding the kinetic interaction between these two molecules.

The pharmacokinetic behaviour of IVM was markedly modified after its co-administration with LPM in rats. Higher IVM plasma availability was observed after IVM + LPM treatment compared to that obtained after treatment with IVM alone. The IVM plasma AUC value was 74.7% higher in the presence of LPM. A high correlation between IVM concentrations attained in plasma and those achieved at most of the tissues of parasite location has been recently demonstrated in cattle (Lifschitz et al 2000). Thus, the enhanced IVM plasma availability obtained after its co-administration with LPM in the current trial may correlate with a greater exposure of tissue-located target parasites to active IVM concentrations. The LPM-induced enhancement of IVM concentration profiles in the liver tissue and small intestine accounted for 60% higher AUC values obtained in these tissues after co-administration with LPM. The faster disposition of LPM, compared to IVM, may account for the existence of a pharmacological interaction only during the first few days post-administration. Therefore, to reach a sustained potentiation of IVM activity, repeated doses of LPM may be necessary. The design of pharmaceutical preparations targeted to achieve sustained LPM concentrations capable of enhancing IVM availability in tissues of parasite location may be a challenge for future research.

Drug molecules with a large molecular weight such as IVM (875) are mainly excreted by bile rather than in urine (Baggot 1977). Large amounts of unchanged IVM are excreted by bile and faeces in cattle (Chiu et al 1990; Lifschitz et al 2000). Additionally, as has been shown for the closely related compound doramectin (Hennessy et al 2000), a significant portion of the systemically available parent drug may be secreted in bile, reabsorbed and enterohepatically recycled, which contributes to its extended residence time in the body of the treated animal. In the current work, a pharmacologically based approach was designed to modify the pharmacokinetic behaviour of IVM. The delayed intestinal transit time induced by LPM may have prolonged the time for reabsorption and enterohepatic circulation of IVM, which was reflected in the enhancement of the plasma availability and decreased body clearance of the drug.

LPM has been classified as both a P-GP substrate and inhibitor molecule (Wandel et al 2002). The lack of central nervous system effects and the restriction of the pharmacological activity of LPM to peripheral tissues may be due to the presence of P-GP in the blood–brain barrier (Schinkel et al 1996; Sadeque et al 2000). IVM has also been reported as a P-GP substrate (Schinkel et al 1994; Didier & Loor 1995, 1996). P-GP-deficient mice (MDR –/–) treated with IVM exhibited increased drug concentrations in different tissues, particularly in the brain (Schinkel et al 1994; Alvinerie et al 1999a). The tissue-specific localization of P-GP at the luminal side of the enterocyte cell and at the biliary canalicular membrane of the hepatocytes contributes to its role as a drug transport system involved in the processes of endo- and xenobiotic excretion from the body (Lin 2003). Several studies

Table 1 Mean (± s.e.m.) IVM pharmacokinetic parameters obtained after its subcutaneous administration (200 $\mu g\,kg^{-1}$) alone or co-administered with LPM to Wistar rats.

Tissue/fluid	t_{max} (h)		C_{max} ($ng\,g^{-1}$)		AUC ($ng\,d\,g^{-1}$)	
	IVM alone	IVM + LPM	IVM alone	IVM + LPM	IVM alone	IVM + LPM
Plasma	6.00 ± 0.00	10.0 ± 2.00	30.2 ± 2.76	35.1 ± 6.82	22.9 ± 2.51	40.0 ± 2.28**
Liver tissue	6.00 ± 0.00	6.00 ± 0.00	256 ± 43.5	277 ± 43.0	177 ± 22.4	285 ± 31.7*
Small intestine tissue wall	6.00 ± 0.00	10.0 ± 2.00	221 ± 50.5	192 ± 28.9	173 ± 30.4	278 ± 11.5*
Large intestine tissue wall	8.00 ± 2.00	16.0 ± 4.00	45.2 ± 0.33	55.9 ± 7.6	71.9 ± 6.99	95.5 ± 11.0
Large intestine lumen content	20.0 ± 4.00	24.0 ± 0.00	819 ± 112	444 ± 145	1089 ± 106	695 ± 93.6*

Values are statistically different from those obtained after the IVM alone treatment at $*P < 0.05$ and $**P < 0.01$.
AUC and C_{max} values in plasma are expressed as $ng\,d\,mL^{-1}$ and $ng\,mL^{-1}$, respectively.

have reported modifications in the plasma kinetic profiles of different drugs given orally as a consequence of interference with the P-GP excretory activity at the intestinal level (Malingre et al 2001; Di Marco et al 2002). The results reported here demonstrate a marked change to the plasma and tissue disposition kinetics of IVM following its parenteral co-administration with LPM. These results differ from those described by Kwei et al (1999) using *mdr1a* deficient mice. These authors found differences in IVM concentrations in intestinal lumen but not in plasma and liver tissue after the intravenous administration of IVM to (+/+) and (–/–) *mdr1* mice (Kwei et al 1999). However, a potential competition between the two co-administered P-GP substrates, LPM and IVM, may account for the pharmacokinetic interaction described here. P-GP may therefore play a relevant role in the bile and intestinal elimination of IVM. The increased IVM concentration profiles found in the liver and small intestine are in agreement with a LPM-induced interference with the P-GP-mediated biliary and intestinal secretion of IVM.

Quantitatively, the major route for the elimination of IVM in the rat is intestinal secretion. The amount of IVM parent drug actively secreted in the small intestinal lumen was five times higher than that eliminated in bile (Laffont et al 2002). The relative involvement of the biliary and intestinal excretion mechanisms for IVM in different species needs to be elucidated. However, the relevance of the intestinal secretion process on the overall excretion pattern of IVM was clearly reflected by the high concentration of the parent drug found in the large intestine luminal content. The peak IVM concentration (C_{max}) in the luminal content of the large intestine was 27-fold higher than that observed in plasma after the administration of IVM alone (Table 1). A marked modification to the pattern of IVM elimination was observed in the presence of LPM. The delayed elimination of IVM after IVM + LPM treatment accounted for the significantly lower concentration ratio between the luminal content and plasma obtained between 6 and 48 h post-treatment (Figure 4). This concentration ratio ranged from 52 (IVM + LPM) up to 237 (IVM alone) at 24 h post-treatment, which clearly indicates that a higher amount of IVM is reaching the luminal content of the large intestine to be excreted by faeces in the treatment without LPM. In humans, mRNA levels of P-GP appear to increase progressively from the stomach to the colonic section of the large intestine (Fojo et al 1987; Fricker et al 1996). However, there is no information available on animal interspecies-related differences in the expression and activity of P-GP along the gastrointestinal tract. A higher level of expression and greater P-GP excretory activity at the enterocyte cell in the distal portion of the gastrointestinal tract may account for the significantly lower IVM concentrations found in the large intestine wall tissue ($P < 0.05$) compared to those measured in the small intestine wall, and for the high amount of IVM recovered in the luminal content of the large intestine.

A family of P-GP homologues has been described in nematodes (Sangster 1994). A higher expression of P-GP was described in an IVM-resistant selected strain of the nematode *Haemonchus contortus* (Xu et al 1998). The combination of IVM or moxidectin with the P-GP modulator agent verapamil increased the antiparasitic efficacy against resistant strains of *H. contortus* in jirds (Xu et al 1998; Molento & Prichard 1999). The co-administration of topically given IVM with verapamil in rats resulted in a 40% enhancement of IVM plasma availability (Alvinerie et al 1999b). Recently, increased moxidectin plasma profiles have been reported after its co-administration with P-GP inhibitor compounds in ruminants. After the co-administration of moxidectin with LPM in cattle (Lifschitz et al 2002) and with the flavonoid quercetin in lambs (Dupuy et al 2003), the systemic availability of the ML compound increased between 46 and 83%, respectively. It is therefore likely that the use of strategies based on the combination of endectocide molecules, such as IVM or moxidectin, and P-GP substrates enhances the availability of the active drug in tissues of parasite location that may increase their anthelmintic efficacy, particularly against the most difficult parasites to be controlled in veterinary and human medicine.

Conclusions

The pharmaco-toxicological effects of any xenobiotic compound are highly dependent on its pharmacokinetic behaviour, including the absorption, tissue distribution,

metabolism and elimination processes. In the last few years it has become apparent that different cell transport proteins play a relevant role in controlling the tissue distribution and excretion of pharmacologically active drugs and metabolites. Increasing attention has therefore been focused on understanding the influence of transport protein systems on the pharmacokinetics and pharmacodynamics of different xenobiotic compounds used in drug therapy in both human and veterinary medicine. The delayed intestinal transit time caused by LPM accounting for an extended plasma–intestine recycling time, and the potential competition between IVM and LPM for the P-GP-mediated bile–intestinal secretion processes may account for the enhanced IVM systemic availability reported here. Further studies are currently being conducted to obtain a better understanding of this type of interaction and to evaluate the practical implication of the co-administrations of P-GP substrates in therapeutics.

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