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Pretreatment with the inducers rifampicin and phenobarbital alters ivermectin gastrointestinal disposition

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The goal of the study was to evaluate the effects of rifampicin (RFP) and phenobarbital (PBT) on the plasma and gastrointestinal disposition kinetics of ivermectin (IVM) subcutaneously administered to Wistar rats. Fifty seven rats were used. Animals in Group I were the noninduced (control) group. Those in Groups II and III received a treatment with RFP (160 mg/day) and PBT (35 mg/day), respectively, both given orally during eight consecutive days as induction regimen. The IVM pharmacokinetic study was started 24 h after the RFP and PBT last administration. Animals received IVM (200 μg/kg) by subcutaneous injection. Rats were sacrificed between 6 h and 3 days after IVM administration. Blood and samples of liver tissue, intestinal wall and luminal content of jejunum were collected from each animal. IVM concentrations were measured by high performance liquid chromatography. IVM disposition kinetics in plasma and tissues was significantly modified by the PBT treatment, but not by RFP. Despite the enhanced CYP3A activity observed after the pretreatment with RPF and PBT, there were no marked changes on the percentages of IVM metabolites recovered from the bloodstream in induced and noninduced animals. An enhanced P-glycoprotein-mediated intestinal transport activity in pretreated animals (particularly in PBT pretreated rats) may explain the drastic changes observed on IVM disposition.

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INTRODUCTION

Ivermectin (IVM) is a broad-spectrum anthelmintic compound extensively used in human and veterinary medicine. IVM is largely excreted in bile and faeces as the parent drug in different animal species (Chiu *et al.*, 1990; Lifschitz *et al.*, 2000). The active intestinal secretion of IVM has been demonstrated using the intestinal close-loop model (Laffont *et al.*, 2002) and the everted gut sac technique in the rat (Ballent *et al.*, 2006).

P-glycoprotein (P-gp), the product of the gene MDR1, plays an important role in the transport of many endogenous and xenobiotic compounds out of the cell in an ATP-dependent process (Lin, 2003). Drug transport by P-gp is recognized as an important determinant for the disposition kinetics of numerous pharmacological agents. Changes on the expression and/or activity of P-gp could modify the patterns of drug absorption, tissue distribution and excretion. Modulation of P-gp activity by the use of specific inhibitors has been performed to assess the contribution of cell transporters in regulating pharmacokinetic interactions for several compounds (Mayer *et al.*, 1997; Song *et al.*, 1999). Similarly, P-gp up-regulation has also been recognized as an important mechanism of drug interaction by reducing intracellular drug concentrations (Lin, 2003).

Drug-drug interactions between P-gp substrates and cytochrome P4503A-related compounds have been investigated. Numerous therapeutic compounds, which were initially described as CYP3A substrates, have been recently described as P-gp substrates or inhibitors because of the strong overlapping substrate specificities between CYP3A and P-gp (Wacher *et al.*, 1995). These overlapped activities may result in the modulation of the oral bioavailability and subsequent changes on the pharmacodynamic effect of various CYP3A/P-gp-related compounds (Yumoto *et al.*, 2001). A concomitant induction of gastrointestinal P-gp and cytochrome P450 isoforms by compounds such as rifampicin (RFP) and dexamethasone has been reported (Yumoto *et al.*, 2001; Schuetz *et al.*, 1996; Salphati & Benet, 1998). Whereas phenobarbital (PBT) is a

well-known cytochrome P450 inducer (Lin *et al.*, 1999), its effect on the intestinal P-gp activity has not been explored.

Ivermectin has been reported as a P-gp substrate (Schinkel *et al.*, 1994, 1996; Pouliot *et al.*, 1997). Likewise, the *in vivo* modulation of P-gp activity by specific inhibitors resulted in marked changes on the IVM disposition kinetics in different animal species (Lifschitz *et al.*, 2004; Ballent *et al.*, 2007; Alvinerie *et al.*, 2008), confirming the relevance of the P-gp-mediated transport on IVM intestinal secretion. However, available information on the effect of inducer agents on the *in vivo* disposition kinetics of therapeutically used P-gp substrates is rather limited. The goal of the study reported here was to evaluate the effects of two prototypical inducer agents (rifampicin and phenobarbital) on the plasma and gastrointestinal disposition kinetics of IVM subcutaneously administered to Wistar rats. Complementary, the influence of both inducer compounds on the liver cytochrome P4503A activity was also assessed.

MATERIALS AND METHODS

Experimental animals, treatment and sampling

Fifty seven male Wistar rats weighing 250–300 g were used in this trial. The management of experimental animals was performed in accordance with institutional and internationally accepted welfare guidelines (American Veterinary Medical Association, 2001). The animals were kept under controlled conditions of temperature and cycles of darkness/light. Rats received food (pelleted feed) and water *ad libitum*. Animals in Group I were the noninduced (control) group. Those in Groups II and III received a pretreatment with RFP (160 mg/day) and PBT (35 mg/day), respectively, both given orally during eight consecutive days as induction regimen. Once the induction period elapsed, four animals from each experimental group were sacrificed. Subcellular fractions from liver tissue were prepared.

The IVM pharmacokinetic study was started 24 h after the RFP and PBT last administration. In fact, the noninduced (control) and RFP and PBT pretreated animals received IVM at 200 μg/kg by subcutaneous (s.c.) injection. The original 1% IVM formulation was diluted in propylene glycol to fit a low dose volume. Within each experimental group, three trial runs were performed. One animal from each group was sacrificed at 6, 12, 24, 48 and 72 h post-treatment in each trial run. Thus, three rats from each experimental group were sacrificed at every sampling time point. Individual samples of blood, liver, wall tissue and luminal content of jejunum were collected from each animal. 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 IVM HPLC analysis.

Preparation of liver microsomes

After sacrifice, the abdomen was opened and the liver was removed. Samples were rinsed with ice-cold KCl 1.15%. All subsequent operations were performed between 0 and 4 °C. Liver samples (6 g) from each experimental animal were cut into small pieces with scissors. Microsomal fractions from liver were isolated by differential ultracentrifugation. Tissue samples were weighted and homogenized using a Potter-Elvenjem tool (four to six passes) with two volumes of ice-cold homogenization buffer (Tris-HCl 20 mM, KCl 1.15%, EDTA 1 mM, pH 7.5). Homogenates were filtered through a hydrophilic gauze, centrifuged at 10 000 ***g*** for 20 min and the resulting supernatant at 100 000 ***g*** for 65 min. The pellets (microsomal preparations) were suspended in a 10 mM Tris-HCl (containing 1 mM of EDTA and 20% of glycerol), frozen in liquid nitrogen and stored at −70 °C until used for incubation assays. An aliquot of the microsomal fraction was used to determine protein content using bovine serum albumin as a control standard (Lowry *et al.*, 1951).

Cytochrome P4503A activity in rat liver microsomes

The response to RPF and PBT treatments was assessed by the quantification of P450-dependent N-demethylase activities towards oleandomycin triacetate (Nebbia *et al.*, 2003), which is believed to be a marker for the expression of CYP450 3A based on molecular models of mammalian enzymes (Lewis, 1996). CYP450 3A activity was determined by aerobic incubations under conditions yielding zero order rates respect to cofactor and substrate concentrations and ensuring linearity with respect to time and protein concentrations. Oxidative P450-dependent N-demethylation activities toward oleandomycin triacetate (0.3 mM), were assayed using the NADPH generating system and 1 mg of microsomal protein per mL of incubation medium. After a suitable incubation time, reactions were quenched with chilled trichloroacetic acid (10%, w/v) and after centrifugation, the amount of the released formaldehyde was determined fluorometrically on an aliquot of the clear supernatant with Nash's reagent (Werringloer, 1978). Incubations of the same substrates with liver microsomes from untreated rats were used as controls.

Ivermectin analysis and validation procedures

The extraction of IVM from plasma, liver and intestinal wall and luminal content was carried out following the technique earlier described by Alvinerie *et al.* (1993) and adapted by Lifschitz *et al.* (2000). Samples including plasma, liver tissue, intestinal wall and luminal content (0.250 mL or g) were fortified with 10 ng of abamectin (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 more for 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 deionised 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, which 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 procedure 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. The measurement of IVM concentrations in plasma and biological tissues was determinated by HPLC (Shimadzu 10 A HPLC system, 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 (5:40:55 v/v/v) pumped at a flow rate of 1.5 mL/min through a reverse phase C_{18} column (Selectosil, Phenomenex, Torrance, 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, intestinal wall 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 1 and 40 ng/mL (plasma) and 1 and 80 ng/g (tissues). 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 rate of eliminations (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 linear regression lines showed correlation rate of eliminations of 0.999. The mean recoveries of IVM were in a range between 74 and 77% for the different tissues analysed. The limit of quantification was established at 1 ng/g or 1 ng/mL for plasma, liver and intestinal wall tissues and contents. CV <8% were collected when the inter-day precision of the chromatographic method was evaluated for the different biological matrices under investigation.

Pharmacokinetic and statistical analyses of the data

The plasma and gastrointestinal tissue concentrations vs. time curves collected 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. 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 & 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 In2/λz. IVM plasma and intestinal tissue concentrations and all the estimated pharmacokinetic parameters are reported as mean ± SD. 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 collected from all experimental groups were statistically compared using ANOVA (Instat 3.0, Graph Pad software Inc.), followed by Bonferroni's test to indicate the level of significance among groups. A value of $P < 0.05$ was considered statistically significant.

RESULTS

Ivermectin was recovered from the plasma and all biological tissues analysed from 6 h up to 3 days postadministration. IVM plasma disposition kinetics was not altered in the RFP-pretreatment group (Group II) compared with control rats. However, the IVM disposition kinetics was clearly modified by the PBT-pretreatment (Group III). The IVM plasma concentration measured at 3 days was 2.9-fold lower in the PBT-pretreatment group compared with the controls. The effects of RFP and PBT on the IVM plasma disposition kinetics are shown in Figs 1 and 2, respectively. The Cmax and *AUC* values measured for IVM in plasma were significantly lower after the PBT-pretreatment compared with those collected in both control and RFP-pretreatment groups. The IVM plasma C_{max} values decreased 54% in PBT-treated compared with control animals. Table 1 summarizes the IVM plasma pharmacokinetic parameters collected for the different experimental groups.

Phenobarbital treatment induced a marked decrease on the IVM accumulation in the intestinal wall. IVM concentrations in the jejunum wall tended to be lower after the RPF pretreatment. The low number of animals used and the high variability observed may contribute to the lack of statistical significance observed after the RPF administration compared with the control group. The IVM availabilities in the jejunum wall, measured as *AUC* values, were 205 (control), 156 (Group II) and 81 ng·g/day (Group III). The ratio between the IVM concentrations measured in the luminal content and intestinal wall tissue (jejunum) was used as an estimator of the P-gp-mediated intestinal secretion of the antiparasitic compound. A significantly higher ratio (119 and 92%) between the IVM *AUC*s measured in the luminal content and intestinal wall was collected after the treatment with RFP and PBT, respectively, compared with that observed in noninduced animals. The IVM

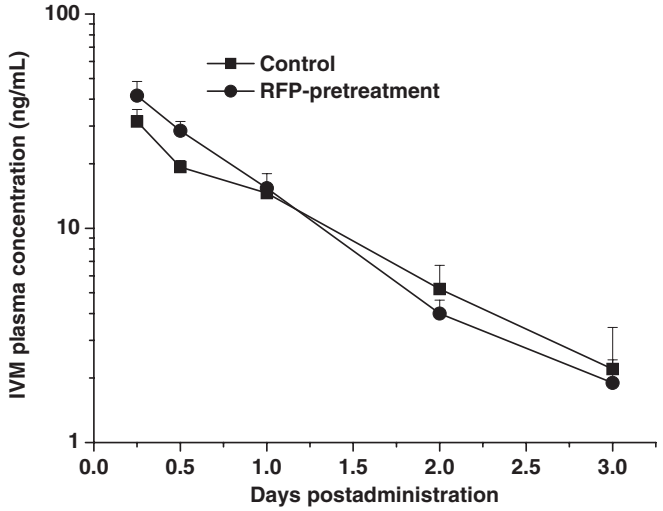

Fig. 1. Mean (±SD) ivermectin (IVM) concentration profiles (ng/mL) measured in plasma after its subcutaneous administration (200 μg/kg) to noninduced (control) and rifampicin (RFP)-pretreated (160 mg/day for 8 days, orally) male Wistar rats.

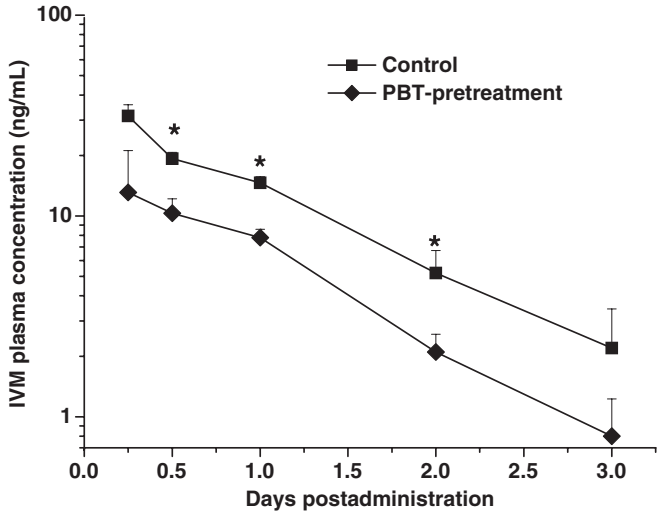

Fig. 2. Mean (±SD) ivermectin (IVM) concentration profiles (ng/mL) measured in plasma after its subcutaneous administration (200 μg/kg) to noninduced (control) and phenobarbital (PBT)-pretreated (35 mg/day for 8 days, orally) male Wistar rats. (*) Values are significantly different from those obtained in control group at $P < 0.05$.

availability in the jejunum wall and the ratios between IVM *AUC* values collected in the luminal content and intestinal wall of PBT and RFP treated compared with control animals are shown in Fig. 3.

Likewise, PBT pretreatment affected the pattern of IVM concentrations in the liver tissue. IVM availability (*AUC* values) in the liver tissue of control animals was 2.4-fold higher than those measured in the liver of PBT treated animals, respectively (Fig. 4). RPF tended to reduce the IVM availability in liver but without reaching statistical significance. The effect of RPF and PBT on the liver microsomal metabolic

Table 1. Comparative ivermectin (IVM) plasma pharmacokinetic parameters obtained after its subcutaneous administration (200 μg/kg) to noninduced (control), rifampicin (RFP)-pretreated (160 mg/day for 8 days, orally) and phenobarbital (PBT)-pretreated (35 mg/day for 8 days, orally) male Wistar rats

	Control	RFP-Pretreatment	PBT-Pretreatment
C_{max} (ng/mL)	31.5 ± 7.42a	41.5 ± 11.7a	15.6 ± 11.8b
AUC (ng·d/mL)	35.3 ± 8.75a	39.2 ± 6.03a	16.2 ± 5.05b
$t_{½el}$ (days)	0.71 ± 0.27a	0.61 ± 0.13a	0.60 ± 0.15a

Data are expressed as mean ± SD ($n = 3$). Values followed by different letter are significantly different at $P < 0.05$.

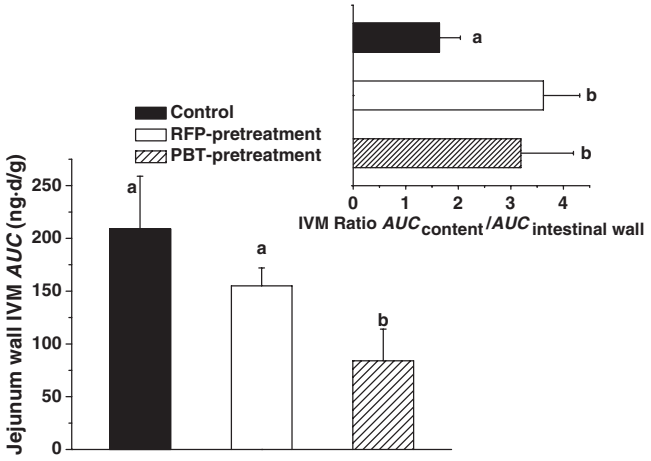

Fig. 3. Ivermectin area under the concentrations vs. time curve (*AUC*) (mean ± SD, $n = 3$) measured in the jejunum wall tissue after its subcutaneous administration (200 μg/kg) to noninduced (control), rifampicin (RFP)-pretreated (160 mg/day for 8 days, orally) and phenobarbital (PBT)-pretreated (35 mg/day for 8 days, orally) male Wistar rats. The insert shows the ratio between IVM *AUC* (mean ± SD., $n = 3$) measured at the luminal content and intestinal jejunum wall used as an estimation of the P-glycoprotein-mediated intestinal secretion. Values lacking a common letter are significantly different at $P < 0.05$.

capacity was also evaluated. The effects of RPF and PBT chronic administration on the CYP3A mediated N-demethylase activities were measured in rat liver. This metabolic activity was between 2.12 (RPF) and 2.95 (PBT) fold higher compared with that collected in liver microsomes from noninduced rats. However, the *in vivo* metabolism of IVM was not modified after pretreatment with the inducers. Unidentified metabolites were detected in plasma after IVM treatment in the different experimental groups. The metabolites percentage from the total amount of the parent drug detected in plasma was calculated. The estimation of the percentage of metabolites recovered in the bloodstream was similar between control and induced rats (Table 2). A comparison of the IVM chromatograms collected after analysis of spiked sample (20 ng/mL) and experimental plasma samples collected at 12 h after IVM administration from noninduced and RFP and PBT treated rats are shown in Fig. 5.

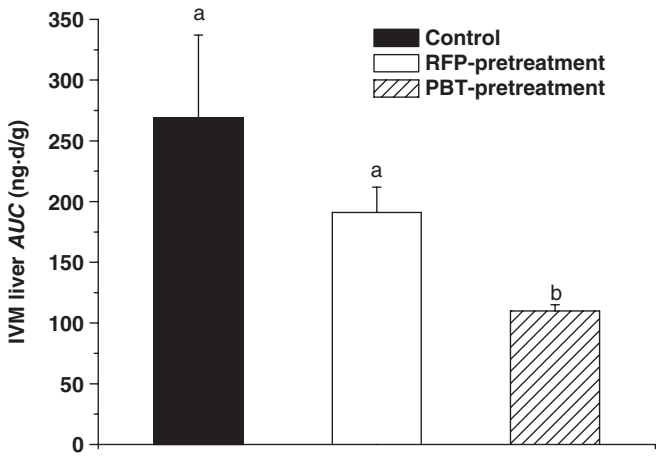

Fig. 4. Ivermectin area under the concentrations vs. time curve (*AUC*) (mean ± SD, $n = 3$) measured in the liver tissue after its subcutaneous administration (200 μg/kg) to noninduced (control), rifampicin (RFP)-pretreated (160 mg/day for 8 days, orally) and phenobarbital (PBT) pretreated (35 mg/day for 8 days, orally) male Wistar rats. Values lacking a common letter are significantly different at $P < 0.05$.

Table 2. Comparative N-demethylase activity measured in liver microsomes and mean percentage of ivermectin (IVM) metabolites recovered in plasma from noninduced (control), rifampicin (RFP)-pretreated (160 mg/day for 8 days, orally) and phenobarbital (PBT)-pretreated (35 mg/day for 8 days, orally) male Wistar rats

	Oleandomycin-triacetate N-demethylase activity (CYP3A subfamily)	Percentage of IVM metabolites*
Control	1.19 ± 0.52a	7.70 ± 4.85a
RFP-pretreatment	2.52 ± 1.00b	5.00 ± 2.45a
PBT-pretreatment	3.51 ± 1.00b	6.30 ± 6.72a

Enzymatic activities (mean ± SD) are expressed in nmol of formaldehyde released per min per mg of microsomal protein ($n = 4$ animals). Values followed by different letter are significantly different at $P < 0.05$.
*The metabolites percentage was calculated from the total amount of the parent drug detected in plasma.

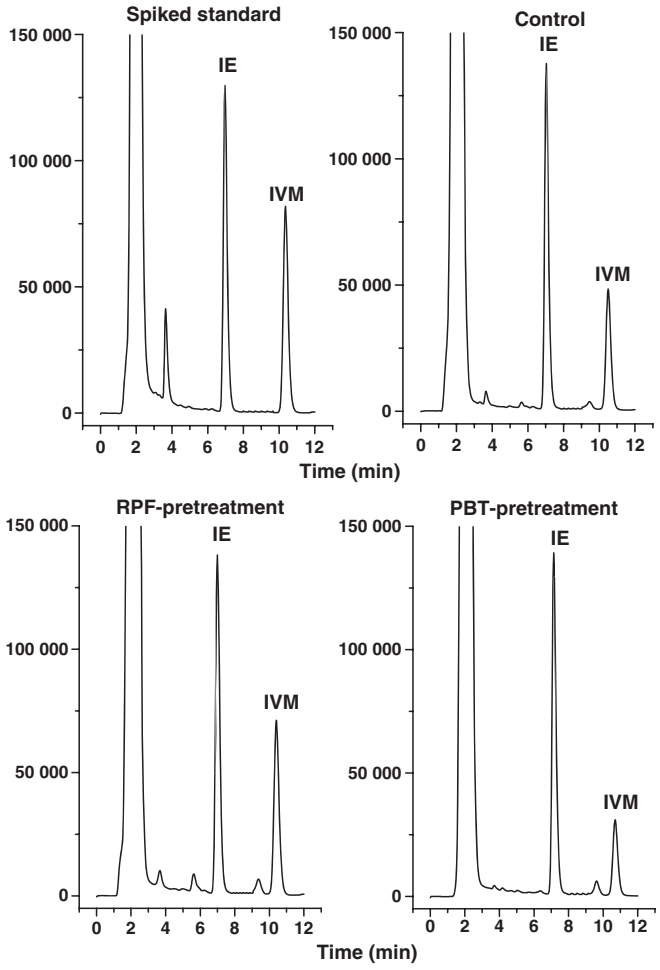

Fig. 5. Comparison of representative IVM chromatograms obtained after HPLC analysis of a spiked plasma sample (20 ng/mL) and experimental plasma samples (collected at 12 h after IVM administration) from noninduced (control), rifampicin (RFP)-pretreated (160 mg/day for 8 days, orally) and phenobarbital (PBT)-pretreated (35 mg/day for 8 days, orally) male Wistar rats. IE: internal standard. Despite the enhanced metabolic activity observed after the pretreatment with RPF and PBT there were no marked changes on the percentages of IVM metabolites recovered from the bloodstream in induced and noninduced animals.

DISCUSSION

P-glycoprotein, a MDR1 gene product, has been demonstrated to be an important determinant of the disposition kinetics of numerous compounds, which has been characterized both under *in vivo* and *in vitro* conditions. Recently, the importance of cell transporter modulation in drug-drug interactions has been recognized (Matheny *et al.*, 2004). *In vitro* approaches have demonstrated that there is a strong overlapping specificity in agents capable of inducing CYP3A and P-gp expression in culture cells. Whereas numerous therapeutic agents can induce P-gp expression under *in vitro* systems, the relevance of these observations to P-gp induction *in vivo* is not entirely clear. Because of the lack of tools and the complexity of the combined transporter-enzyme system, as well as transporter-transporter interplay, it is rather difficult to extrapolate *in vitro* data on transporter-mediated drug interactions to an *in vivo* situation.

The effect of PBT on both the plasma and gastrointestinal disposition of IVM was examined. PBT has been recognized as a CYP3A inducer and increased CYP3A protein concentrations after PBT pretreatment has been reported in cultured rat hepatocytes (Zangar & Novak, 1998). Likewise, PBT modified the hepatic activity of the canalicular multidrug resistance-associated protein 2 (MRP2), another member of the ABC transporter proteins, in isolated perfused livers (Patel *et al.*, 2003). Several antiepileptic drugs, including phenytoin and PBT, have been recently shown to be transported by P-gp in the blood-brain barrier (Löscher & Potschka, 2002). In cell lines, P-gp is strongly up-regulated by many drugs, including antiepileptic drugs such as PBT (Schuetz *et al.*, 1996). Recently, Martin *et al.*

(2008) reported an increase in the P-gp expression at both mRNA and protein levels in hepatic and intestinal cells after incubation with PBT. However, data on the *in vivo* effect of PBT on the P-gp activity are scarce. In the current study, PBT treatment accounted for a marked reduction on IVM availability in the bloodstream and at the gastrointestinal tissues analysed. The IVM *AUC* values were 2.2 (plasma), 2.5 (jejunum) and 2.4 (liver)-fold lower than those measured in noninduced animals. Consistently, C_{max} values were 51% (plasma) and 61% (jejunum wall) lower in PBT treated compared with the controls. The IVM intestinal secretion was increased in the PBT treated animals, as it is shown by the enhancement (93%) of the ratio between the IVM *AUC* values observed in the luminal content and intestinal wall tissue. It was reported that PBT modify the bile flow in rats (Klaasen, 1971). Although the increased bile flow caused by PBT may have influenced the kinetic disposition of IVM, the intestinal secretion plays a greater role than biliary secretion in the overall elimination of IVM in the rat, providing large amounts of active drug to the intestinal lumen and to faeces (Laffont *et al.*, 2002).

Rifampicin is commonly used in different experimental trials as a prototypical inducer of drug-metabolizing enzymes and transporters (Greiner *et al.*, 1999; Sandstrom & Lennernas, 1999; Westphal *et al.*, 2000). Previous studies showed that RFP may act as a powerful inducer of intestinal P-gp both in human duodenal biopsies (Sandstrom & Lennernas, 1999) and in cultured cell lines (Schuetz *et al.*, 1996). In the *in vivo* study reported here, RFP treatment did not produce significant changes on the plasma kinetics of IVM administered by the s.c. route. However, the presence of RFP modified IVM accumulation at intestinal level. At 2 days postadministration, IVM concentrations in the jejunum and liver tissues were 3.5 and 2.3-fold lower in RFP-pretreatment (Group II) compared with control animals, respectively. Consequently, the amount of IVM excreted to the intestinal lumen was significantly increased in the RFP-pretreatment (Group II) compared with noninduced rats. In fact, the ratio between the IVM *AUC* values in the luminal content and intestinal wall was significantly greater after treatment with RFP, suggesting an increment in the IVM active intestinal secretion process. *In vivo* interaction between RFP and digoxin (a P-gp substrate) was previously observed (Greiner *et al.*, 1999), where the *AUC* of orally administered digoxin was significantly lower in the presence of RFP (600 mg/day). However, this effect was negligible when digoxin was administered intravenously. Although the IVM plasma availability was similar in the RFP treated and control animals in the current trial, the IVM intestinal secretion was clearly enhanced likely because of induction of the P-gp-mediated transport occurring after RFP administration (Figs 1 and 3).

Considering the well-established overlapping between CYP3A and P-gp (Schuetz *et al.*, 1996), it is likely that RFP and PBT pretreatments modify both IVM metabolism and transport pathways. In fact, the pretreatment with RFP and PBT markedly increased the CYP450 3A activity in the liver (Table 2). However, previous studies have demonstrated that IVM is poorly metabolized in several animal species and excreted by bile and faeces largely as the parent drug (Chui *et al.*, 1987; Lifschitz *et al.*, 2000). More recent work demonstrated that the drug-drug interaction produced after the co-administration of IVM and ketoconazole (a known inhibitor of both CYP3A and P-gp) in sheep (Alvinerie *et al.*, 2008) and dogs (Hugnet *et al.*, 2007) does not seem to be related to inhibition on IVM metabolism by the antifungal compound. The concentration ratios for IVM parent compound to its metabolites in the bloodstream were equivalent in dogs and sheep receiving IVM alone or co-administered with ketoconozale. The outcome from that work indicates that the increased IVM plasma availability observed after its co-administration with ketoconazole was not because of changes on IVM biotransformation. Instead, a ketoconazole-P-gp interaction may explain the changes observed on IVM disposition in both animal species.

In the experiments reported here, there were some unidentified analytes in the plasma samples collected from IVM treated rats. The major liver metabolite of IVM in rats is a 24-hydroxymethyl-dihydroa-avermectin B1a derivative (Chui *et al.*, 1987). Despite the enhanced CYP3A activity observed after pretreatment with RPF and PBT (induced animals), there were no marked changes on the percentages of IVM metabolites recovered from the bloodstream in induced and noninduced animals (Table 2, Fig. 5). Thus, the potential inductive effect of RFP and/or PBT on CYP3A metabolism in rats does not seem sufficient to explain the altered IVM disposition kinetics observed in the current work. Alternatively, an enhanced P-gp-mediated intestinal transport activity in induced animals (particularly in PBT pretreated rats) may account to explain the drastic changes observed on IVM disposition. Thus, IVM resulted to be an adequate substrate molecule to evaluate the *in vivo* effects of P-gp inducers on its pharmacokinetic disposition following the subcutaneous treatment.

It is likely that the pharmacological implications of the *in vivo* interaction between P-gp and its substrates may differ according to the route of drug substrates administration (Greiner *et al.*, 1999; Ballent *et al.*, 2007). Most studies analyse the local effect (gut lumen) of P-gp activity after oral administration of the drug under assessment. The findings reported here confirm the influence of two different inducer compounds on the gastrointestinal secretion of IVM after its parenteral administration. The clinical significance of transporter-mediated drug interactions for compounds commonly use in human and veterinary therapeutics is a relevant issue. From the data available in the literature, it can be assumed that the magnitude of the changes on drug systemic availability caused by transporter-mediated interactions may be much smaller than those produced by CYP-mediated interactions (Lin, 2007). However, the results collected in this trial clearly demonstrate that changes on drug tissue concentrations caused by modulation of cell transporter activity may be substantially greater than those observed in the bloodstream. The relevance of the induction of transport protein is not only limited to mammals. It has been recently reported that IVM treatment may select for a constitutive or inducible overexpression of at least five P-gp isoforms in adult nematode *Haemonchus contortus* (Prichard & Roulet, 2007), which may have a relevant

impact on the development of parasite resistance to drug action. Considering the role of cellular transporters in numerous drug-drug interactions, the understanding of the molecular basis of the *in vivo* P-gp induction and its clinical implications for human and veterinary therapeutics is being further investigated in our laboratory.

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