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Sex-related differences in the gastrointestinal disposition of ivermectin in the rat: P-glycoprotein involvement and itraconazole modulation

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Abstract

Ivermectin (IVM), a macrocyclic lactone used as antiparasite agent, has been reported as a P-glycoprotein (P-gp) substrate. The participation of P-gp in the IVM excretion process has been previously demonstrated. Sex-related differences in the kinetic behaviour of some macrocyclic lactone compounds have been observed. The aim of this work was to characterize in-vivo the comparative gastrointestinal disposition of IVM in male and female rats. The sex-related influence on the itraconazole (ITZ) modulation of P-gp-mediated IVM intestinal transport was also assessed. Sixty Wistar rats (30 male, 30 female) received IVM alone or co-administered with ITZ. Rats were killed between 6 and 72 h after treatment and blood, gastrointestinal tissues and lumen contents were collected. IVM concentrations were determined by high performance liquid chromatography. Substantial sex-related differences in the IVM disposition kinetics were observed. Higher IVM systemic availability was observed in female rats. The ITZ-mediated modulation of the IVM disposition kinetics had a differential impact between male and female rats. Co-administration with ITZ resulted in a marked increase in the IVM concentrations in the wall tissue from different portions of the gastrointestinal tract of male rats. The presence of ITZ induced drastic sex-related changes on the P-gp-mediated IVM gastrointestinal disposition.

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Introduction

Ivermectin (IVM) is a macrocyclic lactone used worldwide as an antiparasite agent in veterinary and human medicine. Its pharmacokinetic behaviour has been studied extensively in different animal species. The high lipophilicity of IVM has been correlated with its extensive distribution to tissues of parasite location and with prolonged residence in fat-containing tissues in cattle (Lifschitz et al 2000). Large concentrations of unchanged IVM are eliminated by bile and faeces in different animal species (Chiu et al 1990; Lifschitz et al 2000).

P-Glycoprotein (P-gp) is a transmembrane protein associated with a phenotype of multidrug resistance to certain anticancer drugs (Leveque & Jehl 1995). It is expressed in tissues such as the intestine, liver, kidney and placenta (Dantzig et al 2003). The role of P-gp in the pharmacokinetic disposition of different drugs has been demonstrated (Schinkel et al 1995; Van Asperen et al 2000). IVM has been reported as a P-gp substrate (Schinkel et al 1994, 1996; Pouliot et al 1997) and the possible participation of P-gp on the IVM excretion process has been suggested (Laffont et al 2002; Lifschitz et al 2004; Ballent et al 2006). Laffont et al (2002) demonstrated a greater relevance of intestinal secretion compared with biliary excretion for IVM in rats. The co-administration of IVM with loperamide, a P-gp substrate, resulted in a marked decrease in the amount of IVM recovered from the large intestinal luminal content (Lifschitz et al 2004), in addition to other changes in the disposition kinetics of the drug in both cattle (Lifschitz et al 2000) and laboratory animals (Lifschitz et al 2004). Recent experimental work done in-vitro using the everted sac technique demonstrated a significant increase in the IVM accumulation rate in the ileum wall in the presence of itraconazole (ITZ) (Ballent et al 2006).

Sex-related differences in the kinetic behaviour of some macrocyclic lactone compounds have been observed in different animal species. The plasma concentrations of IVM after subcutaneous treatment in cattle were shown to be higher in female compared with male animals (Toutain et al 1997). A similar sex-related trend was observed for selamectin, a structurally related antiparasitic compound, in dogs (Dupuy et al 2004). The mechanism involved in this potential sex-related difference in IVM disposition is not known. One strategy to assess the P-gp function/activity in-vivo is to examine the effects of known P-gp inhibitors on drug pharmacokinetics. The aim of this work was to characterize in-vivo the comparative gastrointestinal disposition of IVM in male and female rats. The sex-related influence on the ITZ modulation of P-gp-mediated IVM intestinal transport was also assessed.

Material and Methods

Experimental animals, treatment and sampling

Sixty Wistar rats (30 male, 30 female), 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 of Animal Care 1980; American Veterinary Medical Association 2000). The animals were housed under conventional conditions with controlled temperature and light/dark cycles. Rats were separated by sex and randomly allocated into four experimental groups of 15 animals each. Female rats in Group A (IVM alone female) and male rats in Group B (IVM alone male) received IVM at 200 $\mu g\,kg^{-1}$ (Ivomec; Merial, Whitehouse Station, NJ) by subcutaneous injection. The original IVM formulation was diluted in propylene glycol to fit the low volume doses. Female rats in Group C (IVM + ITZ female) and male animals in Group D (IVM + ITZ male) received IVM at the same dose rate co-administered with two doses of ITZ (Janssen Animal Health, Beerse, Belgium) (5 mg) diluted in ethanol/propylene glycol by the intraperitoneal route; the first dose of ITZ was administered 12 h before the IVM treatment and the second simultaneously with the IVM administration. Under superficial ether anaesthesia, three animals from each experimental group were killed at 6, 12, 24, 48 and 72 h after treatment. Blood was collected by cardiac puncture in heparinized tubes. Tissue sample collection included liver and gastrointestinal wall tissue, and luminal contents from stomach, duodenum, jejunum, ileum and colon. Blood samples were centrifuged at 2000 *g* for 20 min and the recovered plasma was kept in labelled vials. Plasma and tissue samples were quickly stored at −20°C until analysed.

Analytical procedures

IVM concentrations were determined by high performance liquid chromatography (HPLC) with fluorescence detection using automated solid phase extraction, following a procedure previously described by Alvinerie et al (1993) and modified by Lifschitz et al (2000). Experimental and treated fluid or tissue samples were homogenized and a sample (0.25 g or 0.25 mL) was combined with 10 ng of internal standard (abamectin), 0.25 mL of acetonitrile and 70 μL of water. The mixture was mixed (Multi Tube Vortexer; VWR Scientific Products, West Chester, PA, USA) for 20 min. After mixing, the tissue samples were sonicated for 10 min (Transsonic 570/H; Lab Line Instruments Inc., Melrose park, IL, USA) and the solvent/sample mixture (plasma or tissues) was centrifuged at 2000 *g* for 15 min. The supernatant was manually transferred into a tube and the procedure repeated once for tissue samples. The pooled supernatants obtained were applied to a conditioned Supelclean LC 18 cartridge (Supelco, Bellefonte, PA, USA). After washing with 1 mL water followed by 1 mL water/methanol (4:1, v/v), the cartridges were dried off for 5 min and the sample was eluted with 1.5 mL methanol. The collected 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). The dry residue of the elution was dissolved with 100 μL of *N*-methylimidazole (Sigma, St Louis, MO, USA) solution in acetonitrile (1:1, v/v) and 150 μL of trifluoracetic anhydride (Sigma) solution in acetonitrile (1:2, v/v). After completion of the reaction, 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 (5:40:55, v/v/v) pumped at a flow rate of 1.5 mL min^{-1} through a reverse phase C_{18} column (Selectosil; Phenomenex, Torrance, CA, USA) (5 μm, 4.6 mm × 250 mm). Fluorescence detection (Spectrofluorometric detector RF-10; Shimadzu, Kyoto, Japan) was at an excitation wavelength of 365 nm and an emission wavelength of 475 nm. A complete validation of the analytical procedures for extraction and quantification of IVM in plasma, gastrointestinal wall tissues and luminal contents was performed before starting the analysis of experimental samples. Calibration lines from 1 to 40 $ng\,mL^{-1}$ (plasma) and 1 to 80 $ng\,g^{-1}$ (gastrointestinal wall tissues and contents) were prepared using least squares linear regression analysis, and correlation coefficients (r) were calculated. Linearity was established to express the concentration–detector response relationship, as determined by injection of treated IVM samples. The deviation of the calibration curves from linearity was determined using the analysis of variance test (Instat 3.0; GraphPad Software Inc., San Diego, CA, USA). Drug recovery was estimated by comparison of the peak area from treated plasma and tissues standards at different concentrations with the peak areas resulting from direct injections of standards in methanol. The precision of the method was estimated by processing replicate samples of pooled plasma and gastrointestinal tissue samples containing known IVM concentrations and then calculating the coefficient of variation. The linear regression lines for IVM showed correlation coefficients of > 0.99. The mean extraction recoveries for IVM were $> 70\%$ for the different fluids or tissues. The limit of quantification was established at 1 $ng\,g^{-1}$ or 1 $ng\,mL^{-1}$ for plasma, liver and gastrointestinal wall tissues and contents. The precision of the method showed a

coefficient of variation < 16% for the different biological matrices under investigation.

Pharmacokinetic and statistical analysis

The concentration–time curves obtained for plasma and each gastrointestinal wall tissue and luminal content sample were analysed using the PkSolution 2.0 program (Summit Research Services, Ashland, OH, USA). Pharmacokinetic parameters were determined using a non-compartmental method. The peak concentrations (C_{max}) for each fluid and tissue sample were read from the plotted concentration–time curves. The area under the concentration–time curve from time zero to the last sampling time with a measurable concentration (AUC_{0-last}) was calculated by the trapezoidal rule (Gibaldi & Perrier 1982). Mean pharmacokinetic parameters for IVM obtained after the administration of IVM alone or co-administered with ITZ in both female and male animals were statistically compared using the Student's *t*-test (Instat 3.0; GraphPad Software Inc.). 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

IVM was detected in plasma and in the gastrointestinal wall tissue samples and fluid contents analysed. The co-administration with ITZ resulted in marked changes in IVM pharmacokinetic disposition. IVM plasma concentrations were significantly higher after co-administration with ITZ compared with those obtained after administration of IVM alone. The IVM plasma profiles after both treatments in male and female rats are compared in Figure 1. Mean IVM AUC values in plasma were almost 2-fold greater in the presence of the P-gp modulator. The measurement of IVM in liver tissue was also modified in the presence of ITZ. This effect was particularly relevant in female rats where the AUC values were statistically different compared with those obtained in the female control group. The AUC values obtained for IVM in plasma and liver tissue in animals of both sexes after the IVM alone and IVM + ITZ treatments are compared in Figure 2. The influence of P-gp modulation on IVM disposition from the gastrointestinal tract was assessed in different sections of the digestive tract. The IVM C_{max} and AUC values were significantly increased in the wall tissue of different portions of the gastrointestinal tract of male rats treated with ITZ. Table 1 and Figure 3 summarize the pharmacokinetic parameters obtained for IVM along the gastrointestinal wall tissue after both treatments in male and female rats.

Substantial sex-related differences in IVM disposition kinetics were observed. Higher IVM plasma concentrations were measured in female compared with male rats. IVM plasma concentrations were between 41 and 112% higher in female rats, which accounted for the greater IVM plasma availability compared with that obtained in male rats (Figure 4). IVM availability in the wall tissue of the gastrointestinal tract was markedly greater in female rats. The AUC values obtained for the stomach, duodenum, jejunum and ileum wall tissue of female and male rats are compared in Figure 5. The ITZ-mediated modulation of the IVM disposition kinetics had different impacts on male and female rats. Whereas a significant ITZ-related increase of IVM availability was observed in

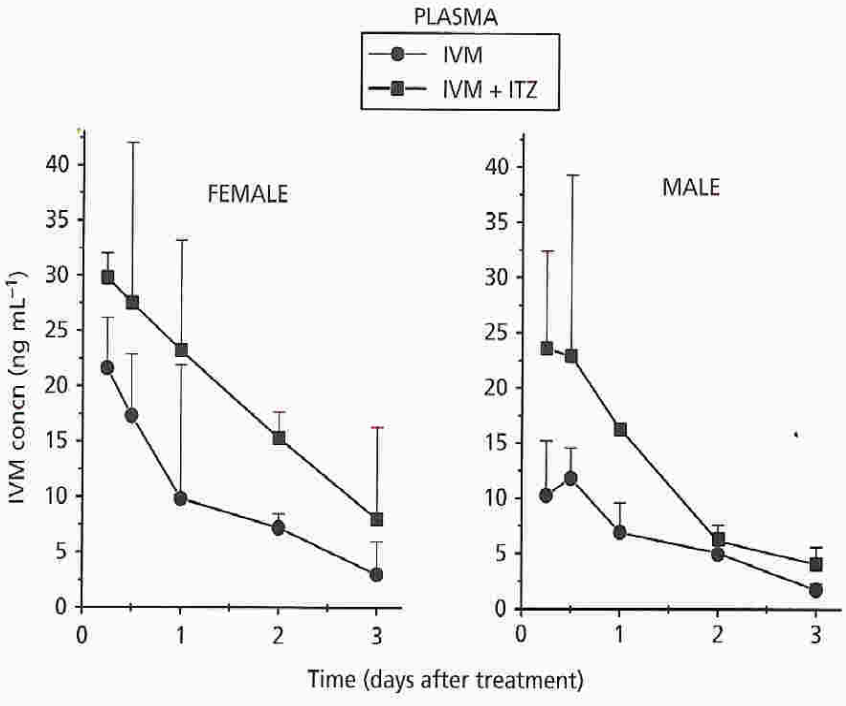

Figure 1 Mean ± s.d. (n = 3) ivermectin (IVM) plasma concentration–time curve obtained after IVM administration (200 $\mu g\,kg^{-1}$) either alone or co-administered with itraconazole (ITZ) to female and male Wistar rats.

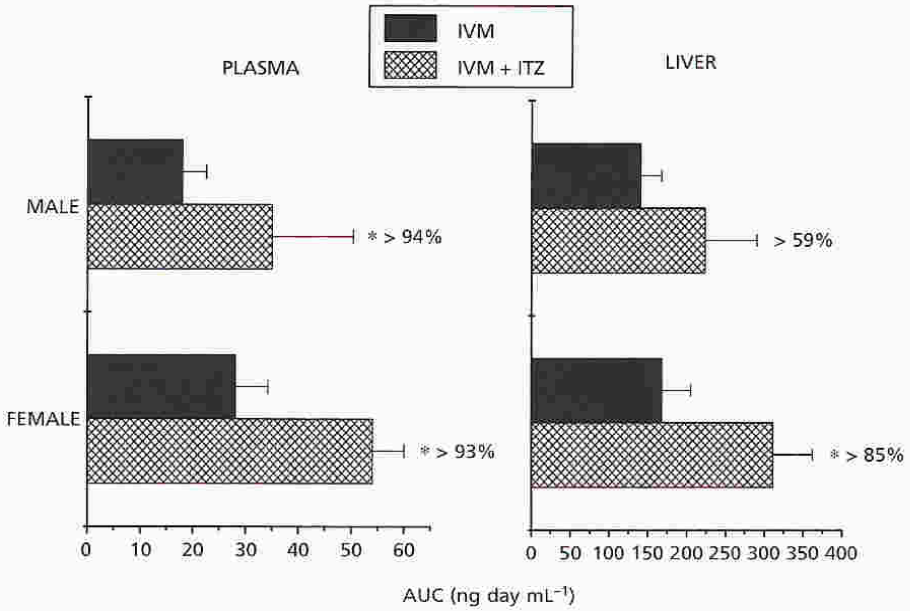

Figure 2 Comparative ivermectin (IVM) (mean ± s.d., n = 3) area under the concentration–time curve (AUC_{0-last}) obtained in plasma and liver tissue after IVM subcutaneous administration (200 $\mu g\,kg^{-1}$) either alone or co-administered with itraconazole (ITZ) to male and female Wistar rats. *$P < 0.05$, statistically different compared with values obtained after the IVM alone treatment.

Table 1 Mean ± s.d. (n = 3) ivermectin (IVM) peak concentrations (C_{max}) measured in different gastrointestinal wall tissues after IVM subcutaneous administration (200 $\mu g\,kg^{-1}$) alone or co-administered with itraconazole (ITZ) to female and male Wistar rats.

Tissue	Female C_{max} ($ng\,g^{-1}$)		Male C_{max} ($ng\,g^{-1}$)	
	IVM	IVM + ITZ	IVM	IVM + ITZ
Stomach	125 ± 22	210 ± 119	57.8 ± 17	235 ± 153*
Duodenum	211 ± 35	233 ± 76	108 ± 35	246 ± 85*
Jejunum	193 ± 105	213 ± 44	139 ± 20	242 ± 23*
Ileum	99 ± 11	201 ± 130	81 ± 9.9	238 ± 42*

*$P < 0.05$, statistically different compared with values obtained after the IVM alone treatment.

all sections of the gastrointestinal tract in males, IVM availability was statistically increased only in the ileum of female rats after co-administration of P-gp modulator. The presence of ITZ significantly enhanced the IVM C_{max} in plasma and the different gastrointestinal wall tissues in male compared with female rats. The percentage increases in C_{max} values after the administration of ITZ in comparison with the control group were 148 ± 93% in male rats and 41 ± 38% in female rats. The effect of ITZ on the relationship between IVM availability in the gastrointestinal wall tissue and luminal contents was also an interesting parameter that revealed sex differences. A significantly greater effect of the P-gp modulator on the ratio between AUC in the gastrointestinal wall and fluid contents was observed in male rats. The ratio between IVM $AUC_{0-12\,h}$ in the gastrointestinal wall and luminal contents obtained for male and female rats in the different sections of the intestine after treatment with either IVM alone or co-administered with ITZ is shown in Figure 6.

Discussion

The wide tissue distribution of different transporter proteins suggests a crucial role in modulating the absorption, distribution and excretion processes for xenobiotic compounds (Leveque & Jehl 1995). The polarized location of P-gp at the apical side of cells such as the hepatocytes and enterocytes facilitates an important role mediating the biliary and intestinal secretion of different compounds (Ayrton & Morgan 2001). Since IVM was recognized as a P-gp substrate, the influence of this transport protein on the pharmacokinetic behaviour of IVM was studied. In-vitro assays performed with tumour cell lines showed that IVM inhibited the P-gp activity and restored the sensitivity to antitumour compounds (Didier & Loor 1996; Pouliot et al 1997). In-vivo studies with P-gp-deficient mice (MDR −/−) treated with IVM lead to increased drug concentrations in different tissues, particularly in the brain (Schinkel et al 1994; Alvinerie et al 1999). A marked change in the plasma and tissue disposition kinetics of IVM was obtained following its parenteral co-administration with loperamide (Lifschitz et al 2004). The effect of ITZ on the pharmacokinetics of IVM, with particular reference to the sex-related differences on gastrointestinal disposition, was evaluated in female and male rats in the present study.

The systemic availability of IVM was significantly increased after its co-administration with ITZ. The mean plasma IVM AUC was almost 1.90-fold greater after

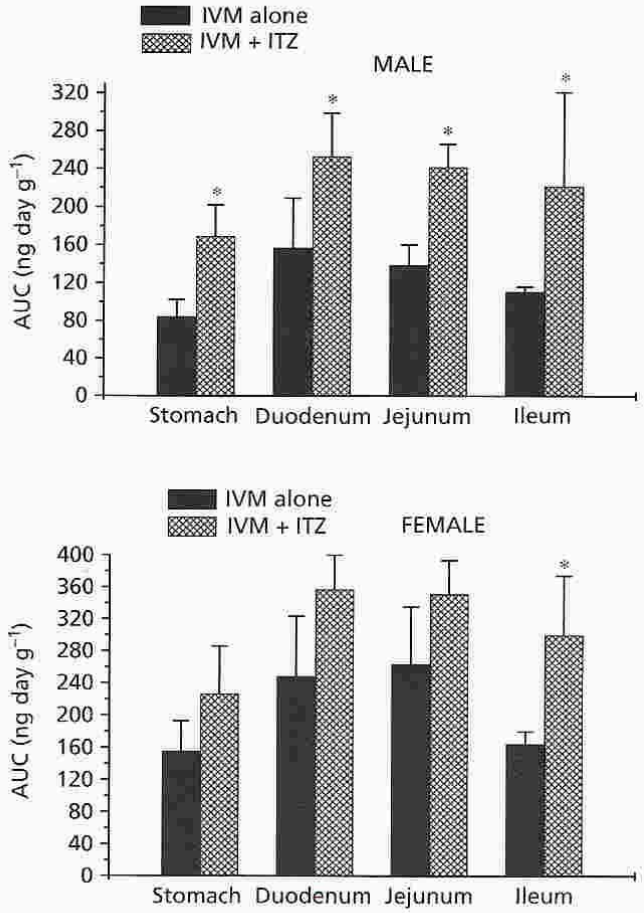

Figure 3 Comparative (mean ± s.d., n = 3) ivermectin (IVM) area under the concentration–time curve (AUC_{0-last}) obtained in the wall tissue of different gastrointestinal tract sections after IVM subcutaneous administration (200 $\mu g\,kg^{-1}$) either alone or co-administered with itraconazole (ITZ) to male and female Wistar rats. *$P < 0.05$, values are statistically different compared with values obtained after the IVM alone treatment.

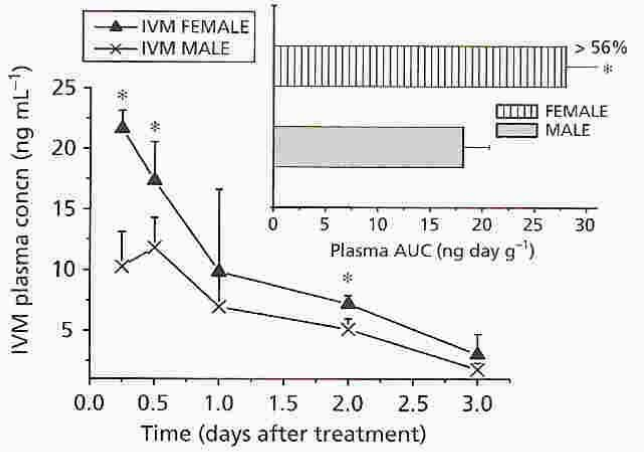

Figure 4 Mean ± s.d. (n = 3) ivermectin (IVM) plasma concentrations–time obtained after its subcutaneous administration (200 $\mu g\,kg^{-1}$) to female and male Wistar rats. The insert shows the comparative plasma area under the concentration–time curve (AUC_{0-last}) for both sexes. *$P < 0.05$, statistically different compared with values obtained in male rats.

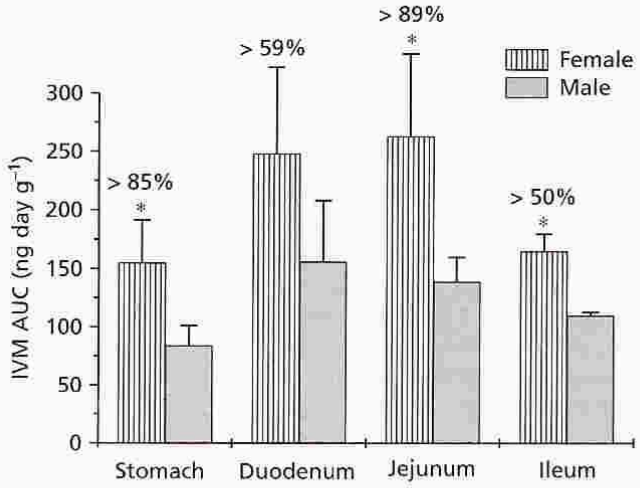

Figure 5 Comparison of area under the concentration–time curve (AUC_{0-last}) (n = 3) obtained in the wall tissue of different gastrointestinal tract sections after subcutaneous administration of ivermectin (IVM) (200 $\mu g\,kg^{-1}$) to female and male Wistar rats. *$P < 0.05$, statistically different compared with values obtained in male rats.

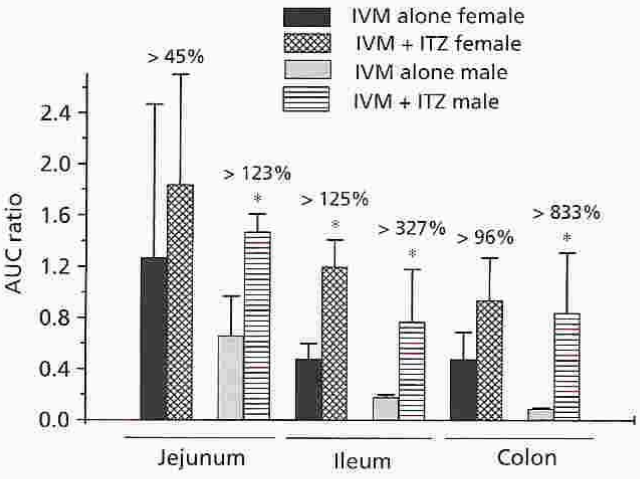

Figure 6 Ratio between ivermectin (IVM) area under the concentration–time curve ($AUC_{0-12\,h}$) in the gastrointestinal wall tissue and the luminal content obtained after IVM subcutaneous administration (200 $\mu g\,kg^{-1}$) either alone or co-administered with itraconazole (ITZ) to female and male Wistar rats. *$P < 0.05$, statistically different compared with values obtained after the IVM alone treatment.

co-administration with the P-gp modulator in animals of both sexes. A large amount of unchanged IVM is excreted by bile (Lifschitz et al 2000) and intestinal secretion (Laffont et al 2002); thus, the enhanced IVM systemic availability obtained after the co-administration with ITZ may reflect a decrease in P-gp-mediated clearance of the antiparasitic compound. The ITZ-mediated increase in the IVM concentration in liver tissue was greater in female rats compared with male rats. As shown in Figure 2, the increase in the IVM AUC in the liver was similar to that observed for plasma in female rats. Conversely, the ITZ-induced increase in liver

availability in male rats was less than that observed in plasma. Intestinal secretion is a major route for the elimination of IVM in the rat. Quantitatively, the amount of IVM parent drug actively secreted in the small intestinal lumen was 5 times greater than that eliminated in bile (Laffont et al 2002). In agreement with recently published data (Ballent et al 2006), the IVM availability measured in the intestinal wall tissue from different sections of the gastrointestinal tract was significantly greater (between 60% and 100%) in the ITZ co-administered group compared with the IVM alone treated group (Figure 3). This effect was particularly relevant in male rats. The faster disposition of ITZ (elimination half-life 9.40 h) (Shin et al 2004) compared with IVM (elimination half-life 30 h) in the rat may have accounted for a pharmacological interaction occurring only during the early hours after their co-administration. Therefore, it is likely that the C_{max} is the most adequate parameter to reflect the IVM and ITZ interaction. The peak IVM tissue concentration in the different portions of the gastrointestinal wall was between 2- and 4-fold higher after the co-administration with ITZ in male rats (Table 1).

Some sex-related pharmacokinetic differences have been described for the class of avermectin antiparasitic compounds. Toutain et al (1997) found a slight sex influence on the availability of doramectin and IVM administered subcutaneously to cattle. Sex had a significant effect on the AUC, which was 10% greater in heifers compared with steers treated with both drugs. The administration of selamectin topically applied to dogs also showed a differential pharmacokinetic pattern between males and females (Dupuy et al 2004). The C_{max} and AUC values for selamectin were 78% and 93% greater in female compared with male Beagle dogs, respectively. A marked sex-related influence on the pharmacokinetics of IVM in rats was observed in the present study. The significantly higher IVM plasma concentration obtained in female rats accounted for the greater systemic availability (56%) compared with that observed in males (Figure 4). At the gastrointestinal level, the IVM availability was also greater in females. The AUC value measured for IVM in the jejunum of female rats was 1.9-fold greater than that observed in males (Figure 5). However, although IVM availability in liver tissue in females (168 ng day g^{-1}) tended to be greater than in males (140 ng day g^{-1}), the difference was not statistically significant.

Cytochrome P450 (CYP) 3A was found to be the main isoform of the CYP enzyme responsible for IVM oxidative metabolism (Zeng et al 1998). Sex-related differences in drug elimination have been reported for many CYP3A substrates, suggesting a differential rate of metabolism between females and males (Hooper & Qing 1990; Hulst et al 1994). Most of the studies in humans suggest that hepatic CYP3A activity may be higher in women compared with men (Cummins et al 2002), while a male-dominant CYP3A isoform expression has been shown in rats (Waxman et al 1995). The lack of sex-influence on the metabolism of IVM was demonstrated in-vitro using liver microsomes from male and female cattle (Dupuy et al 1999). Furthermore, IVM is largely excreted as the unchanged parent drug in bile and faeces (Chiu et al 1990), and a sex-related expression of CYP would not explain the slower IVM clearance observed in female rats in the present work.

Considering that ITZ is a CYP3A inhibitor, drug–drug interaction based on metabolic inhibition cannot be ruled out. However, as the IVM metabolism is extremely low, a potential ITZ inhibition of CYP3A would only play a minor role in the altered IVM disposition kinetics observed in the work reported here. Since IVM is a well established P-gp substrate (Schinkel et al 1994, 1996), the sex-related differences observed in the present study may be owing to a differential activity of this protein transporter. In fact, the effects of ITZ, as a P-gp modulator, on the IVM disposition kinetics were different in female compared with male rats. The observed increase in IVM concentrations in plasma and the different sections of the gastrointestinal tract after co-administration with ITZ was greater in male compared with female rats. Whereas IVM availability in the gastrointestinal wall was statistically enhanced by between 61% and 101% after co-administration with ITZ in male rats, the effect of the P-gp modulator in females was only observed at the ileum wall (Figure 5). Assessment of the increase of the IVM C_{max} values (compared with control) induced by ITZ in rats of both sexes clearly reflects sex-mediated differences on the effect of the P-gp modulator. The ITZ-induced increase of the IVM C_{max} in plasma and gastrointestinal tissues ranged from 112% to 307% in male rats and from 19% to 102% in female rats.

The relationship between the amount of IVM recovered in the wall tissue of different sections of the gastrointestinal tract and their respective luminal contents may be used as an indicator of the intestinal secretion process. A high ratio between IVM level in wall tissue and intestinal luminal content may reflect low P-gp-mediated intestinal secretion activity. The IVM $AUC_{0-12\,h}$ ratio between the gastrointestinal wall and luminal contents for different gastrointestinal sections after the administration of IVM alone or IVM co-administered with ITZ was calculated in animals of both sexes. This AUC ratio increased after the co-administration with ITZ, which reflected inhibition of IVM intestinal secretion by the P-gp modulator. Whereas ITZ produced an increase in this ratio between 45% and 125% in female rats, the effect on male rats was significantly greater, with an increment that ranged from 127% to 833% compared with the control group (Figure 6). Altogether these results may reflect sex-related differences in the IVM clearance mechanism. In addition, the IVM $AUC_{0-12\,h}$ ratio between the gastrointestinal wall and luminal contents may be useful to compare IVM intestinal excretion along the gastrointestinal tract. This ratio was higher in the proximal section of the intestinal tract compared with the caudal segment (Figure 6). A higher increase of the IVM $AUC_{0-12\,h}$ ratio between the gastrointestinal wall and luminal contents was obtained in ileum and colon compared with jejunum in the presence of the P-gp modulator (ITZ), which may agree with the gut regional distribution of P-gp activity involved in digoxin secretion (Stephens et al 2001).

Several hypotheses may be postulated to explain the sex-related pharmacokinetic differences presented in the current study. The available information on this issue is limited and far from conclusive. Salphati & Benet (1998) found that the basal level of P-gp in liver was 40% higher in female rats compared with male rats. A sex difference was not evident for the P-gp expression in the upper duodenum of human intestine (Paine et al 2005). However, other studies have shown slower clearance of a P-gp substrate such as verapamil orally administered in women, suggesting sex-related differences in intestinal P-gp activity (Krecic-Shepard et al 2000). Cummins et al (2002) proposed that sex differences in the oral clearance of dual CYP3A4/P-gp substrates can be attributed to higher P-gp expression in men compared with women. Men seem to have greater activity relative to women for the drug efflux transporter P-gp (Meibohm et al 2002). Although the P-gp expression pattern may differ among species, the greater P-gp activity observed in intestine of men compared with women may agree with the findings obtained in the rat. Since the intestinal secretion process for IVM in rats is quantitatively more relevant than the biliary elimination (Laffont et al 2002), the lower IVM gastrointestinal availability and the greater response to the ITZ co-administration obtained in male rats are in agreement with the hypothesis of greater intestinal P-gp activity in male compared with female animals.

Conclusions

The work described here contributes to the characterization of the sex-related differences involved in the gastrointestinal disposition of IVM in the rat. The ITZ-mediated modulation of P-gp activity accounted for drastic changes in the IVM disposition kinetics, which resulted in marked differences in the systemic exposure according to the sex of the experimental animals. The pharmacological information provided here may be useful in optimizing the use of this antiparasitic compound in both human and veterinary therapeutics. Additionally, the data presented here highlight the need to study sex-related differences in the P-gp expression and activity in liver tissue and along the gastrointestinal tract, which may have considerable physio-pharmacological relevance.

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