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Research Paper

Gender-related differences on P-glycoprotein-mediated drug intestinal transport in rats

Ballent Mariana[a,b]*, Lifschitz Adrián[a,b]*, Virkel Guillermo[a,b], Sallovitz Juan[a], Maté Laura[a,b] and Lanusse Carlos[a,b]

[a]Laboratorio de Farmacología Veterinaria, Facultad de Ciencias Veterinarias, Universidad Nacional del Centro de la Provincia de Buenos Aires (UNCPBA), Campus Universitario, Tandil and [b]Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Tandil, Argentina

Abstract

Objectives Evidence of sex-related differences on drug pharmacokinetics and pharmacodynamics are markedly increasing. The aim of this study was to characterize the influence of gender on P-glycoprotein (P-gp)-mediated drug intestinal transport using two ex-vivo methodological approaches.

Methods To study the comparative tissue uptake of ivermectin, intestinal sacs (distal jejunum/ileum) of male and female Wistar rats were incubated with ivermectin (0.5 μM) (a P-gp substrate) in the presence or absence of PSC833 (10 μM) (a P-gp inhibitor). Additionally, sex-based differences in the bidirectional transport of Rhodamine 123 (Rho 123; 5 μM) incubated either alone or with PSC833 (10 μM) were examined in diffusion chambers.

Key findings The ivermectin accumulation in the everted gut sacs was higher in female compared with male intestine. The presence of PSC833 increased ivermectin accumulation profiles both in male and female rats. However, a greater response to transport modulation was observed in male compared with female animals. Similar results were obtained for Rho 123, where a higher absorption was measured in the intestine of females. PSC833 decreased Rho 123 intestinal secretion in animals of both sexes with a greater inhibition in male.

Conclusions Substantial sex-related differences were observed on the ivermectin and Rho 123 active intestinal transport. Likewise, the PSC833-mediated modulation had a differential impact between male and female animals. Further work is needed to clarify the mechanisms underlying this phenomenon, which may have considerable pharmacological and clinical relevance.

Keywords diffusion chambers; everted gut sacs; ivermectin; P-glycoprotein; sex-related differences

Introduction

Several examples of sex-related differences in the pharmacokinetics and pharmacodynamics of different therapeutically used drugs have been reported. These differences have obvious relevance to the efficacy and side-effect profiles of various medications in human and animals.[1–3] The drug transporter P-glycoprotein (P-gp), the product of the multidrug-resistance gene 1 (MDR1), is one of the most important drug cellular transport systems impacting in both human and veterinary therapeutics. P-gp has a wide tissue distribution and a large number of clinically relevant substrates.[4] Due to the key role played by P-gp on drug disposition, potential sex-mediated differences on its expression and/or activity have been considered as an important determinant for the clinical effectiveness of drug therapy.

Clinically relevant sex differences have been identified for drug metabolism processes and predominantly associated to the sex-specific activity/expression of metabolic enzymes such as cytochrome P450 3A (CYP3A).[5–7] However, recently interest on sex-related differences on P-gp activity and expression has increased.[8–10]

Macrocyclic lactones are antiparasitic drugs widely used in veterinary and human medicine.[11,12] The macrocyclic lactones ivermectin and selamectin are well-known P-gp substrates.[13,14] Gender influence on the in-vivo disposition kinetics of these macrocyclic lactones has been observed in different animal species. In cattle and sheep the plasma levels of ivermectin after its subcutaneous administration were higher in females than males.[15,16] Similar sex-dependent differences were observed for selamectin, a structurally-related

Correspondence: Carlos E. Lanusse, Laboratorio de Farmacología, Departamento de Fisiopatología, Facultad de Ciencias Veterinarias, UNCPBA, Campus Universitario (7000), Tandil, Argentina.
E-mail: clanusse@vet.unicen.edu.ar

*Ballent Mariana and Lifschitz Adrián have equally contributed to this work.

antiparasitic compound, in dogs.[17] Furthermore, the potential involvement of P-gp on the observed in-vivo differences in ivermectin disposition kinetics between male and female rats has been suggested recently.[18] However, the mechanisms underlying those sex-related differences obtained under in-vivo conditions are still unknown. The aim of this study was to characterize the influence of gender on the P-gp-mediated drug intestinal transport. Two ex-vivo methodological approaches, the everted gut sac and the Ussing chamber techniques, were developed to accomplish this aim.

Materials and Methods

Transport studies in everted gut sacs

Gut sac preparation

The everted gut sac method was performed following a technique described by Barthe *et al*.[19] Male and female Wistar rats (250–300 g) were starved overnight. The management of experimental animals was performed in accordance with institutional and internationally accepted welfare guidelines (American Veterinary Medical Association).[20] 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. Under anaesthesia the intestine was rapidly removed and washed with buffer solution 1 mM $NaH_2PO_4.H_2O$; 2.5 mM $CaCl_2.H_2O$; 4.7 mM KCl; 1.1 mM $MgCl_2.6H_2O$; 0.004 mM EDTA; 11 mM glucose; 119 mM NaCl, 25 mM Na_2CO_3 and 0.11 mM ascorbic acid. The entire gastrointestinal tract was removed rapidly from rats and the ileum-caecum valve identified. The first 30 cm of intestine from the ileocaecal valve were used to perform the experiments (distal jejunum/ileum). The intestine was immediately placed in warm (37°C) oxygenated (O_2/CO_2, 95%/5%) TC199 medium (Sigma-Aldrich, Gillingham, Dorset, UK) and then gently everted over a glass rod 2.5 mm in diameter. One end was clamped and tied with a silk braided suture before filling it with medium at 37°C using a 1-ml plastic syringe. The intestinal segment was then sealed with a second tie using a braided silk suture. Sacs (5 cm in length) were placed in individual incubation chambers containing 6 ml pregassed oxygenated media at 37°C.

Incubation assays

To study the comparative tissue uptake of ivermectin in the presence or absence of the P-gp modulator agent, ivermectin (0.5 μM) and PSC833 (10 μM) were added to the chambers. Sacs corresponding to the intestinal segment of distal jejunum/ileum of male and female rats were incubated in a water bath for 10, 30, 60 or 90 min. The solution was maintained at 37°C with O_2/CO_2 (95%/5%) throughout the experiment. At the defined time points, sacs were removed, washed in buffer solution, blotted dry and weighed. Samples were conserved at −20°C until analysis.

Gut sac viability

To verify the integrity of the gut sacs, glucose concentrations were measured in the incubation medium and in the sac contents using a commercial test (Wienerâ, Argentina). As glucose is actively transported by the small intestine, healthy and metabolically active sacs that are not leaking will concentrate glucose in the serosal medium. The sacs were incubated in TC199 medium into the chambers in the absence and presence of ivermectin (0.5 μM) and PSC833 (10 μM), maintaining the same conditions as the experimental assay. The gut sacs were incubated from 30 to 90 min. Samples of incubation medium and content of the sacs were collected and stored until glucose determination by spectrophotometry. Glucose determination was carried out as follows: 20 μl sample was incubated at 37°C for 10 min with a reagent which consisted of 50% distilled water, 5% 4-aminofenazona 25 mmol/l solution in buffer Tris 0.92 mol/l; 5% phenol solution 55 mmol/l and 0.3% glucose oxidase (1000 U/ml)/peroxidase (120 U/ml). Finally, the absorbance was measured in a spectrophotometer at 505 nm.

Ivermectin analysis

The measurement of ivermectin concentrations in the wall of intestinal sacs was carried out by high performance liquid chromatography (HPLC; Shimadzu 10A HPLC system, Shimadzu Corp. Kyoto, Japan). The ivermectin extraction from the intestinal wall and HPLC analysis was performed following the technique described by Alvinerie *et al*. [21] and adapted by Lifschitz *et al*.[22] Abamectin was used as internal standard. Samples of intestinal wall were fortified with 10 ng abamectin and 0.25 ml acetonitrile was added to each sample. The preparation was mixed (Multi Tube Vortexer, VWR Scientific Products, West Chester, PA, USA) over 15 min, sonicated in a 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. The supernatant was applied to a conditioned Strata C18-T cartridge (Phenomenex, Torrance, CA, USA). The cartridge was flushed with 1 ml water and 1 ml water/methanol (4 : 1). The compounds were eluted with 1.5 ml 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). Derivatization was initiated by adding 150 μl trifluoroacetic anhydride (Sigma Chemical, St Louis, MO, USA) solution in acetonitrile (1 : 2). After completion of the reaction (<30 s), a 100-μl sample of this solution 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 through a reverse phase C_{18} column (Selectosil, Phenomenex, Torrance, USA; 5 μm, 4.6 × 250 mm). Ivermectin 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 ratio between ivermectin and abamectin peak areas was used to estimate ivermectin 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 ivermectin in the intestinal wall 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 0.016 and 2.5 nmol/g. Linearity was determined by injection of ivermectin spiked standards (plasma and tissues) at different concentrations (three replicates). Calibration curves were established using least squares linear regression analysis and correlation coefficients (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 ivermectin standards in methanol. The limit of quantification was established as the lowest concentration measured with a recovery higher than 70% and a CV <16%. The linear regression lines showed correlation coefficients of >0.99.

Intestinal transport assays: studies into the Ussing chamber system

The transepithelial transport of Rhodamine 123 (Rho 123) in male and female rat intestine was examined using the diffusion chamber method. Male and female Wistar rats were allowed free access to water and food before the beginning of the experiment. Under anaesthesia, the entire gastrointestinal tract was removed rapidly and the lumen of the intestine was rinsed with ice-cold buffer solution. Intestinal segments corresponding to ileum ($n = 4$ from 4–5 rats) of male and female rats were used for each set of experiments. Each segment was cut open along the mesenteric border and the resulting flat sheets were mounted into Ussing chambers that provided and exposed a tissue area of 0.85 cm^2. Both mucosal (M) and serosal (S) sides were filled with 11 ml pre-warmed and oxygenated Krebs buffer (pH 7.4), which was maintained at 37°C. To ensure oxygenation and agitation, a mixture of 95% O_2 and 5% CO_2 was bubbled through each compartment. Measurement of transepithelial electrical resistance values was conducted before the beginning and at the end of the transport studies to access the integrity of the intestinal tissue. After a 20 min equilibration period, Rho 123 at a final concentration of 5 μM was added to the M or S chambers. For inhibition studies, PSC833 (10 μM) was added both in the donor and acceptor sides. Samples volume of 1 ml was taken from the acceptor chamber at intervals of 30 min.

Rhodamine 123 analysis

Samples for transport studies in the Ussing chambers were mixed with 2 ml Krebs buffer solution to reach a final volume of 3 ml. Calibration curves for Rho 123 were performed in a range between 0.12 and 125 pmol/ml (three replicates). The concentration of Rho 123 was measured by a fluorescent spectrophotometer RF-5301PC (Shimadzu Corporation, Kyoto, Japan) set at an excitation wavelength of 485 nm and an emission wavelength of 520 nm.

Values of unidirectional transepithelial effective permeability (P_{eff}), given as cm/s, for each chamber over each 30 min flux period, were calculated using the following equation:

$$P_{eff} = (dC/dt).[1/(A.C_o)] \quad (1)$$

were dC/dt is the appearance rate on the receiving compartment, calculated from the slope of the concentration versus time curve over a time period between 30 to 180 min, A is the exposed area of the tissue in the Ussing chamber, and C_o is the initial drug concentration in the donor compartment. The efflux ratio was calculated as follows:

$$\text{Efflux ratio} = \frac{\text{mean } P_{\text{eff S-M}}}{\text{mean } P_{\text{eff M-S}}} \quad (2)$$

Statistical Analyses

Mean data for ivermectin and Rho 123 obtained after their incubation alone or with PSC833 in the intestine of male and female rats were statistically compared using the Student's *t*-test (Instat 3.0, Graph Pad software Inc., San Diego, USA). The assumption that the data obtained after both treatments had the same variance was evaluated. A nonparametric Mann-Whitney test was used where significant differences among standard deviations were observed. A value of $P < 0.05$ was considered significant.

Results

Everted gut sac technique

The ability of everted sacs to concentrate glucose by active transport across the intestine wall was used as an indicator of tissue viability. The ratios between the glucose concentrations measured in the sac content and incubation medium of male and female everted sacs incubated with ivermectin either alone or with PSC833 increased gradually from 1 (time 0) to 1.31 (30 min) up to 1.84 at 90 min incubation, indicating an adequate maintenance of the intestinal viability during the whole experimental period.

To investigate the sex influence on the ex-vivo ivermectin intestinal secretion in male and female Wistar rats, ivermectin was incubated for up to 90 min in the presence or absence of PSC833. Ivermectin was gradually accumulated in the intestinal wall of the sacs during the whole experimental period, both in male and female animals. However, marked sex-related differences were observed on ivermectin accumulation in the everted gut sacs. A higher ivermectin accumulation was measured in female compared with male intestine. At 60 min, the accumulation of ivermectin in female, expressed as amount/cm^2, was 2-fold that in male. The accumulation of ivermectin in the intestinal sacs at different incubation times is shown in Figure 1.

The ivermectin accumulation in the everted sacs of male and female rats was significantly higher after its incubation with PSC833 compared with incubation with ivermectin alone. However, a greater response to the modulation was observed in male sacs compared with female (Figure 2). While at 60 min the accumulation of ivermectin in male sacs incubated with PSC833 increased 141% compared with control sacs, the increment with respect to its controls in

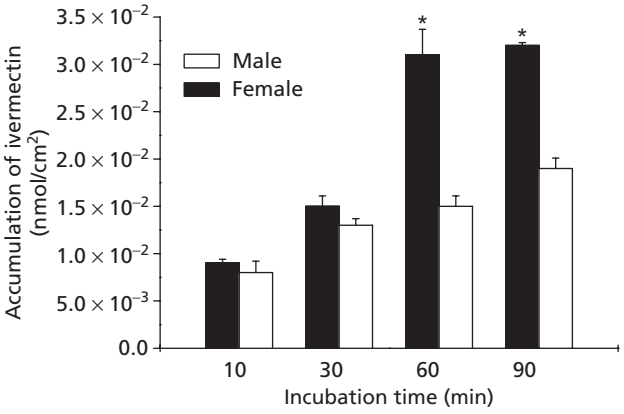

Figure 1 Comparative ivermectin accumulation into the wall of everted guts sacs from male and female Wistar rats. The gut sacs were incubated with ivermectin alone (0.5 μM). $n = 4$. *Values are statistically different between male and female at $P < 0.05$.

females was only approximately 14%. Furthermore, this observation was reflected in the drug intestinal accumulation rate obtained in animals from both sexes. The ivermectin accumulation rate values, expressed as nmol/min/g, in the intestinal sac of females were 4.16 ± 0.31 (control) and 5.41 ± 0.79 (PSC833) whereas the rate values in males was enhanced from 2.88 ± 0.21 (control) to 5.56 ± 0.44 (PSC833).

Ussing chamber assays

To study the mechanisms underlying sex-related differences in drug transport, transepithelial transport of Rho 123 from mucosal to serosal (M–S) and serosal to mucosal (S–M) directions across the ileum of male and female Wistar rats, was examined using the Ussing chamber technique. Transepithelial electrical resistance (Rt) measurement was used as an estimator of intestinal viability (Figure 3). A maximum decrease of 30% of the initial Rt measurements was fixed as an acceptance criteria. The slow fluctuation of Rt values obtained along the whole experimental period in each working day confirmed the maintenance of the functional activity and integrity of the intestinal epithelia.

Rho 123 intestinal absorption (mucosal to serosal side) was higher in female compared with male rats. The amount of Rho 123 at 180 min incubation was 108 ± 18.5 pmol for females and 53 ± 6.50 pmol for males ($P < 0.05$). On the other hand, the intestinal secretion of Rho 123 (serosal to mucosal side) was higher in male compared with female rats. The accumulated amount of Rho 123 in the mucosal side after 180 min incubation was 1.80-fold in the males ($P < 0.05$). Figure 4 shows the absorptive and secretory (bidirectional) transport of Rho 123 across the intestine of male and female rats and the flux obtained in both sexes. Gender-related differences in the transport of Rho 123 were reflected in the effective permeability (P_{eff}). The P_{eff} obtained for mucosal to serosal side transport was 85% higher in female (1.94×10^{-6}) compared with male rats (1.05×10^{-6}). However, the P_{eff} that reflected the secretory activity of the intestine for Rho 123 was 94% greater in males. Principally, the presence of PSC833 affected the secretion process. PSC833 decreased the secretion of Rho 123 in the intestine obtained from animals of both sexes. This effect was notably higher in male compared with female rats, where the P_{eff} (S–M) was reduced from $4.55 \times 10^{-6} \pm 9.63 \times 10^{-7}$ to $7.87 \times 10^{-7} \pm 1.39 \times 10^{-7}$ (males) and from $2.44 \times 10^{-6} \pm 4.47 \times 10^{-7}$ to $1.94 \times 10^{-6} \pm 1.84 \times 10^{-7}$ (females). The efflux ratio (P_{eff} S–M/P_{eff} M–S) obtained for Rho 123 incubated alone or in the presence of PSC833 in male and female rat intestine is shown in Figure 5.

Discussion

Although sex-related differences should be analysed for other ABC transporters, the in-vivo data on pharmacokinetic or toxicological gender-related differences are mostly available for P-gp substrates. The work reported here was undertaken to evaluate sex-related differences on the active P-gp-mediated intestinal transport in rats using recently validated ex-vivo techniques. Several in-vitro approaches have been established to assess the role of the specific proteins involved in the intestinal drug transport processes. However, the assessment of the correlation between quantitative information obtained in-vivo with that provided by in-vitro models is critical. The everted gut sac technique has been widely validated as a simple and accurate method to characterize intestinal transport of different drugs.[19,23] Recent ex-vivo work done in our laboratory using the everted gut sac technique demonstrated a marked increase on the ivermectin intestinal accumulation and serosal transfer rates in the presence of different generations of P-gp inhibitors.[24] In this study, sex-dependent differences on the active intestinal transport of ivermectin were observed using the everted gut sac technique in the rat. The ivermectin concentration measured in the distal jejunum/ileum tissue wall was significantly higher in female than in male intestinal sacs (Figure 1).

The importance of studying sex-based differences is enhanced by the increasing data on gender variation on drug efficacy and toxicity profiles.[1] Regarding gender influence on veterinary therapeutics, sex-related pharmacokinetic differences have been described for the antiparasitic compounds belonging to the avermectin family. A comparative study of the pharmacokinetics of doramectin and ivermectin in cattle has shown that the plasma disposition kinetics of these compounds differs between male and female animals.[15] In addition, Ndong *et al*. [16] reported sex-related differences in the bioavailability of ivermectin in Senegalese sheep, where the area under the curve (AUC) was 69% higher in females than males. The administration of selamectin, an avermectin topically applied to dogs, also showed a differential pharmacokinetic pattern between male and female, where systemic drug exposure was 93% higher in female compared with male Beagle dogs.[17] Recently, the comparative gastrointestinal disposition of ivermectin in female and male rats was characterized.[18] Significantly higher ivermectin plasma concentrations obtained in female rats accounted for the higher systemic availability (56%) compared with that observed in males. At the gastrointestinal level, the ivermectin availability was also higher in females. The AUC values measured for ivermectin in the jejunum (wall tissue) of female rats were 1.9-fold those

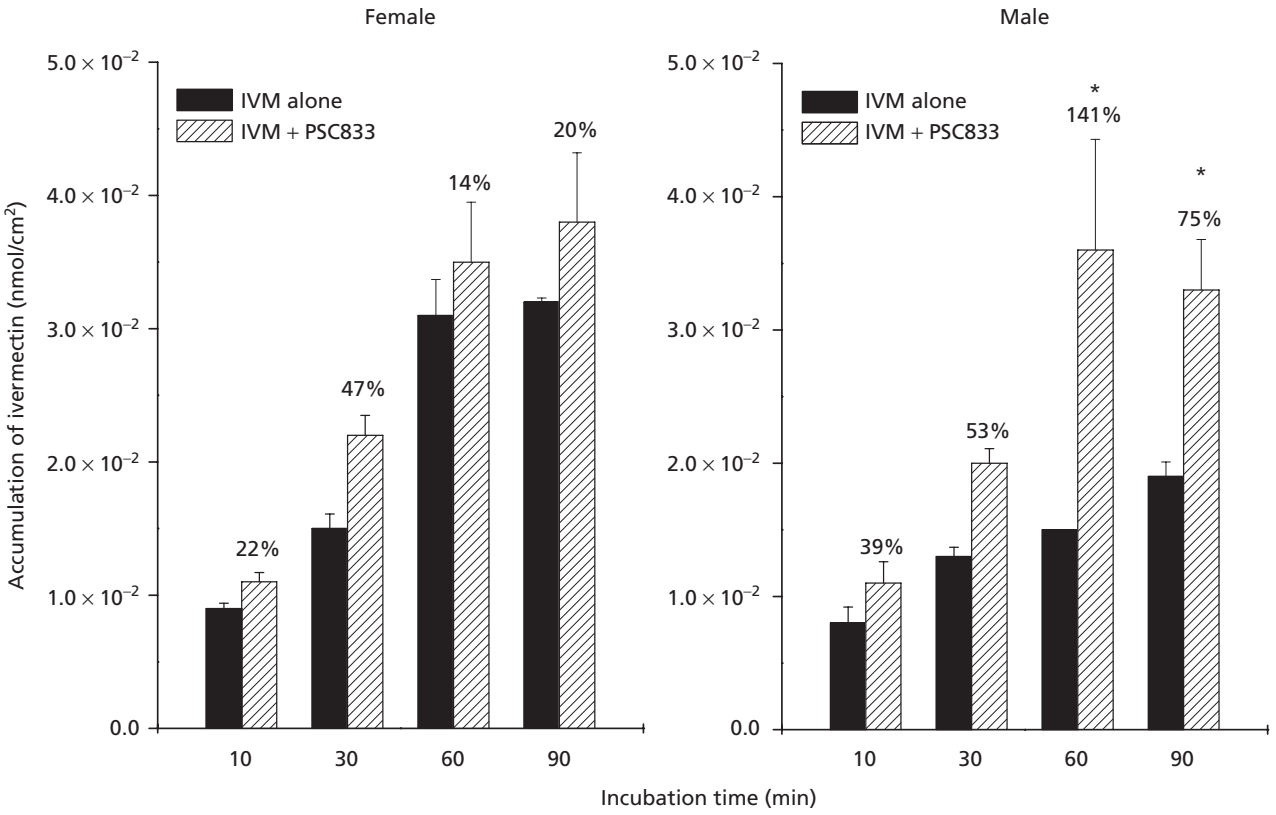

Figure 2 Accumulation of ivermectin into the wall of everted guts sacs from male and female Wistar rats obtained after its incubation either alone or with PSC833. Ivermectin (IVM) 0.5 μM, PSC833 10 μM. *Values are statistically different at $P < 0.05$.

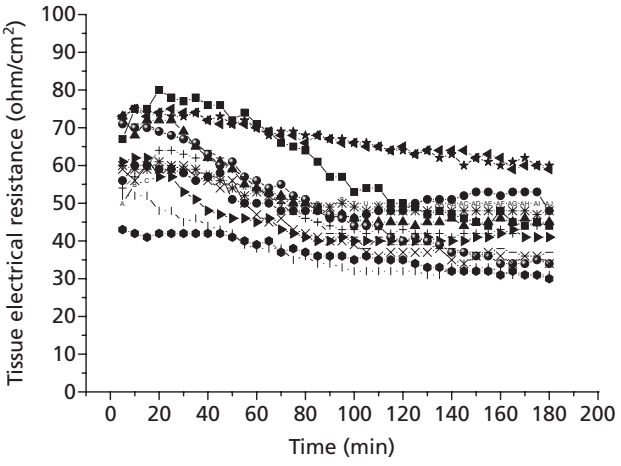

Figure 3 Baseline of transepithelial electrical resistance in rat intestine measured in the Ussing chamber system. The data illustrates on the viability of intestinal tissue over time in different experimental days (each plot corresponds to the tissue resistance values obtained for one specific working day).

observed in males.[18] Interestingly, the itraconazole-mediated in-vivo modulation of P-gp activity mediated affected ivermectin disposition kinetics in a different manner in male and female rats.[18] Itraconazole induced an increase of the ivermectin peak concentration value in plasma and gastrointestinal tissues ranging between 112–307% in male rats. However, this enhancement resulted in a range between 19 and 102% only in female animals.[18] The potential gender differences on P-gp activity at the gastrointestinal tract may explain the results obtained *in vivo*.[18] In agreement with our previous in-vivo results, the response to the P-gp modulation with PSC833 was higher in the everted gut sacs of male compared with female animals (Figure 3). Such a finding confirmed the correlation of these results with those obtained under in-vivo conditions. Recently, it was shown that ivermectin may act as a breast cancer resistance protein (BCRP) inhibitor in culture cell systems.[25,26] Although the in-vivo/ex-vivo interaction of ivermectin with BCRP can not be ruled out, P-gp appeared as the ABC transporter playing the major role in ivermectin transport and body disposition. Further studies are needed to elucidate the relevance of BCRP-mediated interactions on the ivermectin elimination process in different species.

To clarify the sex influence on the P-gp-mediated intestinal transport, the efflux of Rho 123, a well-known P-gp substrate, was investigated in diffusion chambers. The Ussing chamber technique has been previously validated to assess transmembrane transport of different xenobiotics.[27] In veterinary therapeutics, the Ussing chambers have been used to estimate the P_{eff} for drugs administered to horses and to predict the extent of their oral absorption.[28] In this study, the measurement of the epithelial electrical resistance at the beginning and during the incubation period indicated an adequate maintenance of the intestinal functionality and integrity. In agreement with

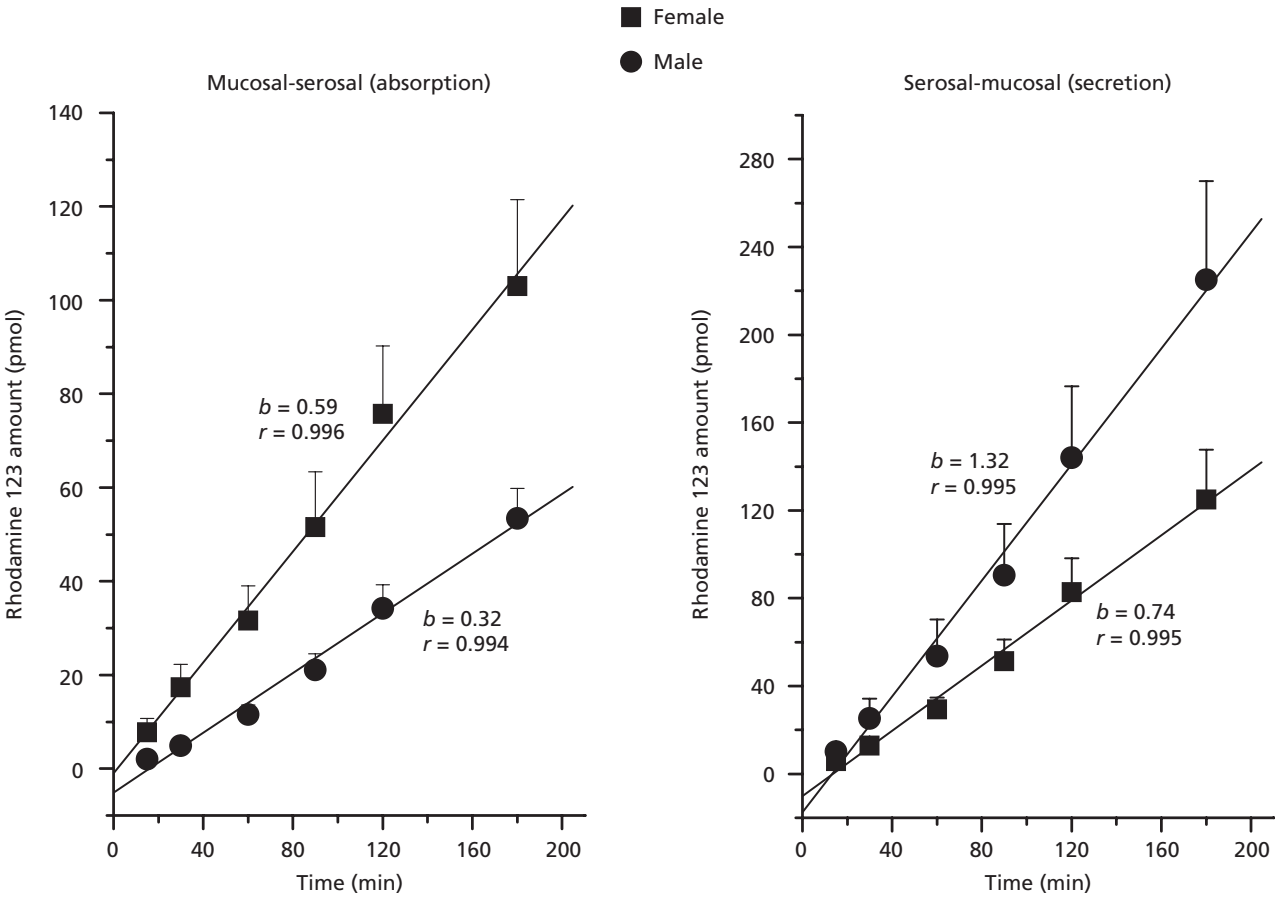

Figure 4 Absorptive and secretory transport of Rhodamine 123 across the intestine of male and female Wistar rats. Passage was measured in the mucosal or serosal compartments of the Ussing chambers. Coefficient of determination (r) and slope (b) values were obtained from lineal regression analysis. Each value is an average of at least eight measurements.

the data obtained for the everted gut sac technique, marked sex-related differences on drug transport were also observed with the diffusion chamber technique. The rate of intestinal absorption of Rho 123 was 1.69-fold higher in female than male rat intestine. However, the Rho 123 intestinal secretion (serosal to mucosal transport) was 1.78-fold higher in male than female, suggesting a greater P-gp activity in male intestine (Figure 4). The presence of PSC833 accounted for a marked reduction of Rho 123 secretion, particularly in male intestine where the efflux ratio decreased from 4.33 (Rho 123 alone) to 1.51 (Rho 123 + PSC833) (Figure 5). Therefore, as occurred with the everted gut sacs, the response to the P-gp inhibitor was higher in male than in female rats. If these findings are associated to a higher P-gp activity in males compared with females, the presence of a P-gp inhibitor such as PSC833 may account for a greater impact by reducing the intestinal P-gp efflux activity in both ex-vivo approaches under study. Considering the well-established overlapping between CYP3A and P-gp, a difference in the metabolism pattern between sexes may explain the observed differences.[29] However, previous studies have demonstrated that ivermectin was poorly metabolized in several animal species and excreted by bile and faeces largely as the parent drug.[22,30] More recent work demonstrated that drug–drug interactions

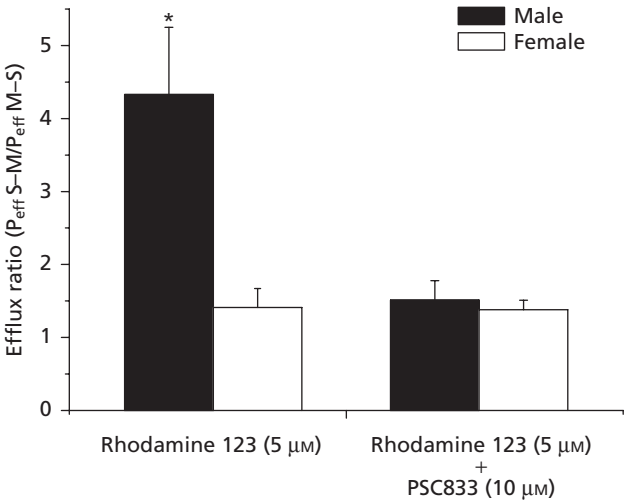

Figure 5 Comparative efflux ratio of Rhodamine 123 across the intestine of male and female Wistar rats after its incubation either alone or with PSC833. Efflux ratio: P_{eff} S–M/P_{eff} M–S, where S is serosal side, M is mucosal side, P_{eff} is effective permeability. Values are expressed as mean ± SEM. Each value is an average of at least eight determinations. *Values are statistically different at $P < 0.05$.

produced after the co-administration of ivermectin and ketoconazole (a known inhibitor of both CYP3A and P-gp) in sheep and dogs did not seem to be related to inhibition of ivermectin metabolism by the antifungal compound.[31,32] In the case of the ex-vivo assay with the Ussing chamber, Rho 123 is suitable to evaluate P-gp activity in intestine due to it has not been described as CYP3A substrate.[33]

The greater P-gp activity observed in male compared with female intestine in the current trials may be based on gender differences on the P-gp expression pattern. However, the available information on this issue is limited and far from conclusive. Schuetz *et al*. [2] reported that men may have a significantly higher level of P-gp compared with women. Potter *et al*. [34] reported that men may have higher enterocyte P-gp content than women. In contrast, Paine *et al*. [10] did not find differences between the sexes in P-gp expression in the upper duodenum. For other ABC transporters, such as BCRP, there was a similar expression in the small intestine of animals of both sexes, but a significant higher expression of BCRP in liver of males compared with females has been reported.[35] Recently, a detailed study was conducted on the ABC transporters expression along the rat intestine. An increase on P-gp expression from proximal to distal intestinal segments was observed in males. In females, the expression was more variable without a clear trend and with a minor relative increase compared with that observed in male animals. However, no significant gender-specific differences were observed in the P-gp expression pattern.[36] The high inter- and intravariability observed in the transporter expression studies, the limited measurements performed only in selected portions of the intestine, and the differences observed between protein and mRNA levels, may have contributed to explain the inconsistencies observed on the available data. This current work has deepened the understanding on the intestinal activity of P-gp in both sexes and further correlation with the expression pattern under standardized experimental conditions is needed.

The clinical consequences of this gender difference should be carefully evaluated. Several studies have indicated differences in toxicity of antiretroviral drugs between man and woman, with an increased toxic risk for women.[37] Although the weight of evidence suggests that in a large majority, sex-based toxicological variation is largely mediated via differences in hepatic enzyme activity, the role of drug transporters is increasingly considered as a potentially relevant factor to explain such differences between men and women.[38,39] The calcium channel blocker verapamil showed a slower clearance in women after its oral administration, supporting sex-based differences in the intestinal CYP3A and/or P-gp activity.[40] In veterinary therapeutics, the clinical implications of potential gender differences remain unknown and are a relevant open field for further research work.

Altogether, our in-vivo and in-vitro results were in agreement with the hypothesis of a greater intestinal P-gp activity and/or expression in male compared with female animals. Finally, sex-associated differences in transporter activity and expression should be discussed considering both the molecular basis and the observed differences in systemic drug exposure. Further work is needed to clarify the mechanisms underlying this phenomenon, which may have considerable pharmacological and clinical relevance.

Conclusion

The work reported here contributes to a better understanding of the physio-pharmacological implications of P-gp on drug transport at intestinal level. The PSC833-mediated modulation of P-gp activity accounted for changes in ivermectin intestinal accumulation and in marked differences between male and female rats, which further contributed to the data obtained previously *in vivo*. Furthermore, the Ussing chamber technique was validated, resulting in a useful method to study the mechanism involved in intestinal drug transport and to confirm the gender influence in drug efflux. Increasing attention on this topic could lead to better efficacy and safety outcomes for both existing and new drugs in human and veterinary medicine.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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