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P-glycoprotein in sheep liver and small intestine: gene expression and transport efflux activity

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The role of the transporter P-glycoprotein (P-gp) in the disposition kinetics of different drugs therapeutically used in veterinary medicine has been demonstrated. Considering the anatomo-physiological features of the ruminant species, the constitutive expression of P-gp (ABCB1) along the sheep gastrointestinal tract was studied. Additionally, the effect of repeated dexamethasone (DEX) administrations on the ABCB1 gene expression in the liver and small intestine was also assessed. The ABCB1 mRNA expression was determined by real-time quantitative PCR. P-gp activity was evaluated in diffusion chambers to determine the efflux of rhodamine 123 (Rho 123) in the ileum from experimental sheep. The constitutive ABCB1 expression was 65-fold higher in the liver than in the intestine (ileum). The highest ABCB1 mRNA expression along the small intestine was observed in the ileum (between 6- and 120-fold higher). The treatment with DEX did not elicit a significant effect on the P-gp gene expression levels in any of the investigated gastrointestinal tissues. Consistently, no significant differences were observed in the intestinal secretion of Rho 123, between untreated control (P_{eff} S-M = $3.99 \times 10^{-6} \pm 2.07 \times 10^{-6}$) and DEX-treated animals (P_{eff} S-M = $6.00 \times 10^{-6} \pm 2.5 \times 10^{-6}$). The understanding of the efflux transporters expression and activity along the digestive tract may help to elucidate clinical implications emerging from drug interactions in livestock.

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INTRODUCTION

P-glycoprotein (P-gp), a member of the adenosine triphosphate (ATP)-binding cassette (ABC) transporter family, plays a relevant role regulating pharmacokinetic interactions of several compounds (Mayer *et al.*, 1997; Song *et al.*, 1999). Physiologically, P-gp acts as an efflux pump in different tissues such as intestine, liver, kidney, and blood–brain barrier for different chemically unrelated compounds. Thus, changes in P-gp expression and activity may modify the patterns of drug absorption, tissue distribution, and excretion.

Whereas the basal P-gp gene expression in the apical membrane of polarized cells of different organs, including the intestine, liver, and kidneys, has been widely investigated in humans and laboratory animals (Thiebaut *et al.*, 1987), the knowledge regarding the constitutive intestinal P-gp expression and its efflux activity in ruminant species has not been described. Recently, sheep P-gp was cloned and expressed in canine culture cells (Zahner *et al.*, 2010). Strong evidence shows that expression and activity of efflux proteins can be inhibited or induced by dietary components, hormones, and several xenobiotics (Schinkel *et al.*, 1996; Schuetz *et al.*, 1996; Salphati & Benet, 1998; Huang *et al.*, 2001; Martin *et al.*, 2008). Whereas, the *in vivo* inhibition of P-gp by specific substances has been assessed to study the contribution of this transporter on the kinetic disposition of antiparasitic drugs in sheep (Molento *et al.*, 2004; Ballent *et al.*, 2007; Alvinerie *et al.*, 2008; Lifschitz *et al.*, 2010), the effect of the *in vivo* induction on P-gp expression and function has not been investigated.

Dexamethasone (DEX), a synthetic glucocorticoid derivative, is widely used in livestock species for its anti-inflammatory and immunosuppressant effect. Regarding transporter proteins such as P-gp, the *in vivo* effect of DEX on its expression has been demonstrated in laboratory animals (Salphati & Benet, 1998; Yumoto *et al.*, 2001). However, the observed interspecies divergences after different *in vivo* induction schemes require additional information on veterinary target species. Thus, within an overall research program aiming to elucidate the physiopharmacological role of protein transporters in the ruminant

gastrointestinal tract, the aim of the study reported here was as follows: (i) to characterize the basal expression pattern of P-gp in sheep liver and small intestine and (ii) to assess the effect of repeated DEX administrations on the *in vivo* P-gp expression and its correlation with the *ex vivo* P-gp intestinal transport activity.

MATERIALS AND METHODS

Experimental animals, treatment, and sampling procedures

The study was conducted in 10 male Corriedale (25–30 kg) clinically healthy and parasite-free sheep. The animals were treated with levamisole, and the parasitological status of the animals was checked by worm egg per gram counts (epg). The same experimental animals have been used before to study the effect of DEX on the expression of metabolic enzymes (see Maté *et al.*, 2012). 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. The animals were randomly allocated into two experimental groups of five sheep each. The control group remained without any treatment. The DEX-treated group received a daily dosage of 3 mg/kg body weight of dexamethasone sodium phosphate (Dexashock 20™, John Martin, Buenos Aires, Argentina) by the intramuscular route for seven consecutive days. Animals from both experimental groups were sacrificed 24 h after the last administration, and livers as well as intestinal segments from duodenum, jejunum, and ileum were harvested. For the liver, a piece of tissue of the caudate lobe was collected and washed in ice-cold KCl solution (1.15% w/v). In the case of the small intestine, segments were excised and perfused with ice-cold (0.9% w/v) saline solution. The intestine was cut lengthwise, and the intestinal mucosa was collected by scraping with glass slides on ice. Aliquots (about 50 mg each) were placed immediately in cryotubes, snap-frozen in liquid nitrogen, and stored at −80 °C until analysis.

Isolation of total RNA and reverse transcription

Total RNA was isolated from about 30 mg of frozen control and DEX-treated sheep liver samples using Pure Link™ RNA Mini kit (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. For the intestinal tissues, the total RNA was extracted using the Trizol reagent according to the manufacturer's protocol (Invitrogen). Briefly, 1 mL of Trizol was added to an aliquot (50 mg) of intestine and homogenized manually several times using a 1-mL syringe. Then, samples were purified with a phenol–chloroform extraction process. To check for RNA integrity and DNA contamination, 5 μL of extracted RNA was run on a SYBR safe (Invitrogen), stained 0.8% agarose gel at 80 V for 60 min. Total RNA purity and concentration were determined spectrophotometrically by absorbance at 260 and 280 nm after dilution of the sample 1:500 in RNase-free water. In all samples, the 260/280 ratio was ≥ 1.8. The reverse transcription was performed with 2 μg of total RNA in a final volume of 20 μL by using the High Capacity cDNA Reverse Transcription kit (Applied Biosystems, Foster City, CA, USA) following the manufacturer's procedure. The reaction was performed in a water bath for 10 min at 25 °C, 120 min at 37 °C, and finally 5 sec at 85 °C for enzyme inactivation. Complementary DNA was stored at −20 °C until use.

Real-time quantitative PCR

The full-length cDNA clone of sheep (*ovis aries*) P-gp has been recently published by Zahner *et al.* (2010). *Ovis aries* mRNA sequence of the target genes was obtained from the GenBank Web site (http://www.ncbi.nlm.nih.gov). Primer sequences used for P-gp expression and quantification analysis were designed by using Primer Express™ Software 2.0 (Applied Biosystems, Carlsbad, CA, USA) and subjected to primer test analyses to exclude dimmer synthesis (Primer Test Document application in Primer Express™ Software; Applied Biosystems). Primer sequences, GenBank accession numbers, and product sizes used for real-time PCR analysis are summarized in Table 1. Each primer set was optimized in a 200- to 500-nM range to identify the concentration that provided the highest sensitivity. Real-time quantification was carried out in an ABI Prism 7500 Real Time PCR System (Applied Biosystems). The reaction mixture included 10 μL of PCR SYBR Green Master Mix 2x (Applied Biosystems), 2 μL of each primer set (250 nM), 1 μL of cDNA diluted 1:250 and 7 μL of water to obtain a final volume of 20 μL. Amplification was carried out in a 96-wells plate. The Real-time PCR was run under the following thermal profile: 50 °C for 2 min and 95 °C for 10 min (holding stage), 40 cycles of 95 °C for 15 sec, and 60 °C for 1 min (cycling stage). Validation curves were performed with decreasing amounts of a cDNA pool diluted at 5-fold intervals to evaluate the real-time PCR efficiency. Standard curves with $-3.6 < \text{slope} < -3.1$ and precision (r^2) higher than 0.985 were considered as acceptable as it is recommended in the ABI Prism 7500 Real Time PCR System guidelines (Applied Biosystems S.A.). Beta-actin was chosen as the reference gene because its amplification efficiency was approximately equal to the target gene and no differences between control and DEX-treated groups were observed in its gene expression. Slope and

Table 1. GenBank accession numbers, sequences of primer sets used for real-time PCR and the respective product sizes

Gene name	GenBank accession number	5′-3′ primer sequence	Product size (bp)
β-actin	NM_001009784	f: gtcgacaccgcaaccagtt r: aagccggccttgcacat	85
P-gp	NM_001009790.1	f: gagagggacgcacttgcat r: caagtctgcgttctggatggt	80

P-gp, P-glycoprotein; f, forward; r, reverse.

Table 2. Validation parameters for the real-time PCR analysis of the studied genes in sheep liver and small intestine

Gene	Tissue	Slope	r^2	E_x	Dynamic range*
ß-actin	Liver	−3.28	0.998	2.02	0.0016–1
ß-actin	Small intestine	−3.36	0.998	1.98	0.0016–1
P-gp	Liver	−3.16	0.996	2.07	0.0016–1
P-gp	Small intestine	−3.24	0.990	2.03	0.0016–1

E_x, PCR efficiency ($E_x = 10^{-1/\text{slope}}$); P-gp, P-glycoprotein.
*Range of target RNA serial dilutions.

r^2 values, real-time PCR efficiency, and dynamic range for each gene in each tissue are shown in Table 2.

Transport studies in diffusion chambers

Sheep intestinal segments corresponding to the ileum of DEX-treated and control animals were used for each set of experiments. Segments were cut along the mesenteric border, and resulting flat sheets were mounted into Ussing chambers with exposed area of 1 cm^2. Both mucosal (M) and serosal (S) compartments were filled with 11 mL of prewarmed and oxygenated Krebs buffer (pH 7.4), which was maintained at 37 °C. To ensure oxygenation and agitation, each compartment was aerated with carbogen (95% O_2 and 5% CO_2). Measurement of transepithelial electrical resistance (R_t) values was conducted prior to the beginning and at the end of the transport studies to verify the integrity of the intestinal tissue. A maximum decrease of 30% of the initial R_t measurements was fixed as an acceptance criterion. After a 20-min equilibration period, Rho 123 in a final concentration of 5 μM was added to the M or S compartment of the chambers. To further verify the P-gp-mediated transport of Rho 123, inhibition studies were addressed using IVM (10 μM) as a P-gp-specific inhibitor. Samples of 1 mL were taken from the acceptor chamber at intervals of 30 min and then mixed with 2 mL of buffer solution, to reach a final volume of 3 mL prior to quantification of Rho 123. Calibration curves for Rho 123 were performed in a range between 0.12 and 125 pmol/mL (three replicates). The concentrations of Rho 123 were measured by a fluorescent spectrophotometer RF-5301PC (Shimadzu Corporation, Kyoto, Japan) set at an excitation wavelength of 485 nm and at an emission wavelength of 520 nm.

Intestinal efflux analysis

Values of unidirectional transepithelial effective permeability (P_{eff}) (cm per sec) for each chamber over each 30-min flux period were calculated using the following equation:

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

The appearance rate on the receiving compartment is dC/dt, calculated from the slope of the concentration vs. time curve over a time period of 240 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:

$$\text{Efflux ratio} = \frac{\text{mean } P_{eff}\text{S-M}}{\text{mean } P_{eff}\text{M-S}}$$

Statistical analysis

The relative quantification of mRNA expression was carried out by using the $\Delta\Delta C_t$ method (Livak & Schmittgen, 2001). Data are expressed as mean ± SD of $\Delta\Delta C_t$ (three replicates) and presented as fold change compared with the untreated control group (with a mean value of 1 arbitrarily assigned). Values were compared using Mann–Whitney U-test. For permeability studies, mean P_{eff} determined from at least seven measurements were statistically compared using Student's t-test (Instat 3.0, GraphPad software Inc., San Diego, USA). A value of $P < 0.05$ was considered statistically significant.

RESULTS

P-gp mRNA analysis

Gene expression profiles were measured by using a real-time PCR approach. The stability of β-actin, used as housekeeping gene, was confirmed as no statistical differences were observed in the mRNA expression levels between experimental groups in all the analyzed tissues. Specific mRNA for the analyzed genes was detected in the gastrointestinal tract of sheep. A higher ABCB1 expression was measured in the liver compared with that measured in the intestine. The hepatic P-gp mRNA in

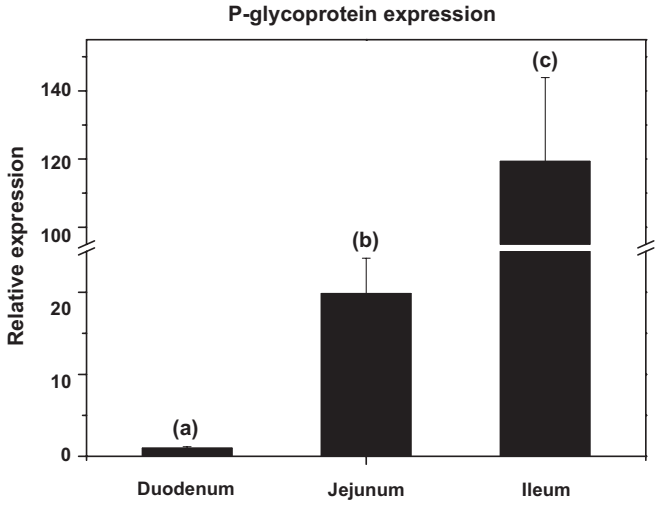

Fig. 1. Comparative ABCB1 mRNA expression profiles (n = 5, three replicates) measured by Real-time PCR in different sections of the small intestine of untreated control sheep. Data are expressed as fold change compared with the duodenal segment (arbitrarily set to a mean value of 1). Different letters represent statistical differences at $P < 0.05$.

control animals was 65-fold higher than that obtained in the ileum ($P < 0.05$) (data not shown). At intestinal level, the analysis of the ABCB1 mRNA abundance showed marked differences along the small intestine (Fig. 1). The expression of ABCB1 mRNA was significantly greater in the ileum compared with the other intestinal segments (119- and 6-fold higher than duodenum and jejunum, respectively).

The repeated administrations of DEX to sheep did not elicit a significant effect on ABCB1 gene expression in the liver (Fig. 2). Interestingly, the administration of DEX tended to decrease the ABCB1 mRNA expression in the intestine. In DEX-treated sheep, the ABCB1 mRNA expression was 0.41 ± 0.14- (duodenum), 0.61 ± 0.09- (jejunum), and 0.71 ± 0.20-fold (ileum) lower compared with the controls, without showing significant differences.

Diffusion studies

The Ussing chamber technique was validated to study the transepithelial transport of Rho 123 from mucosal to serosal (M-S) and serosal to mucosal (S-M) directions in the ileum of DEX-treated and untreated control animals. An intestinal efflux of Rho 123 was confirmed. The efflux ratio (P_{eff} S-M/P_{eff} M-S) value was 5.32 ± 2.13 (Fig. 4). The P_{eff} of Rho 123 from serosal to mucosal (P_{eff} S-M) (secretion) was $3.99 \times 10^{-6} \pm 2.07 \times 10^{-6}$, whereas the P_{eff} M-S (absorption) was $0.66 \times 10^{-6} \pm 0.47 \times 10^{-6}$ cm per sec. No significant differences were measured in the Rho 123 secretion across the intestine of DEX-treated animals (P_{eff} S-M $= 6.00 \times 10^{-6} \pm 2.5 \times 10^{-6}$) compared with the controls (Fig. 3a). The efflux ratio value in the intestine of DEX-treated sheep was 8.03 ± 2.61 (Fig. 3b). The presence of IVM, used as specific P-gp inhibitor, significantly reduced ($P < 0.01$) the efflux of Rho 123 to 1.13 ± 0.97 that confirms its strong interaction with the intestinal P-gp in the sheep (Fig. 4).

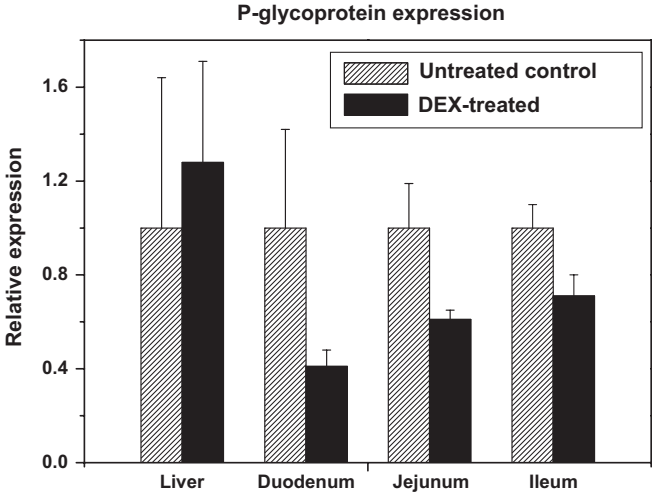

Fig. 2. ABCB1 mRNA expression profiles ($n = 5$, three replicates) measured by Real-time PCR in the liver and small intestine of control and dexamethasone-treated (DEX-treated) sheep. Data are expressed as -fold change compared with untreated control animals (arbitrarily set to a mean value of 1).

DISCUSSION

In the current work, the constitutive expression of the multidrug transporter P-gp in the liver and small intestine of sheep was characterized. P-gp was detected at mRNA level in all analyzed gastrointestinal tissues. The interspecies differences in veterinary medicine lead to the necessity of conducting studies in specific animal species to obtain accurate information. In that context, the anatomo-physiological complexity of the ruminants highlights the importance to characterize the constitutive expression of P-gp along the gastrointestinal tract of sheep. Significant differences in the expression profiles of

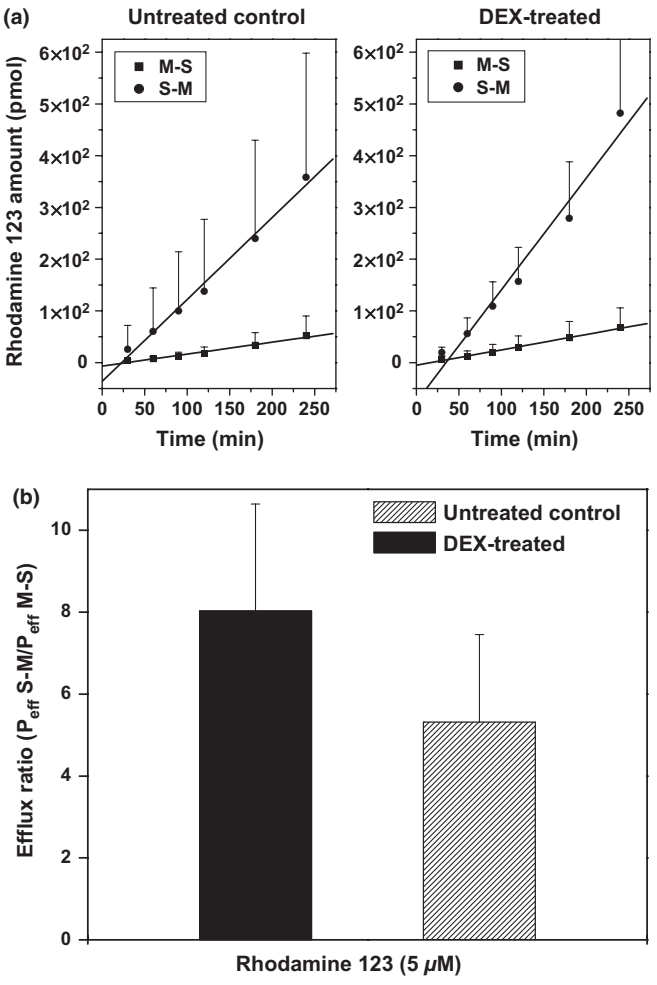

Fig. 3. (a) Rhodamine 123 (Rho 123) P-gp-mediated intestinal transport (mean ± SD) across the ileal segment of untreated control and dexamethasone-treated (DEX-treated) sheep. Passage was measured in the mucosal (S-M) or serosal (M-S) compartments of the Ussing chambers. The efflux ratio (P_{eff} S-M/P_{eff} M-S) for Rho 123 in the intestine of untreated control and DEX-treated animals is shown in (b). Each value is an average of at least seven measurements.

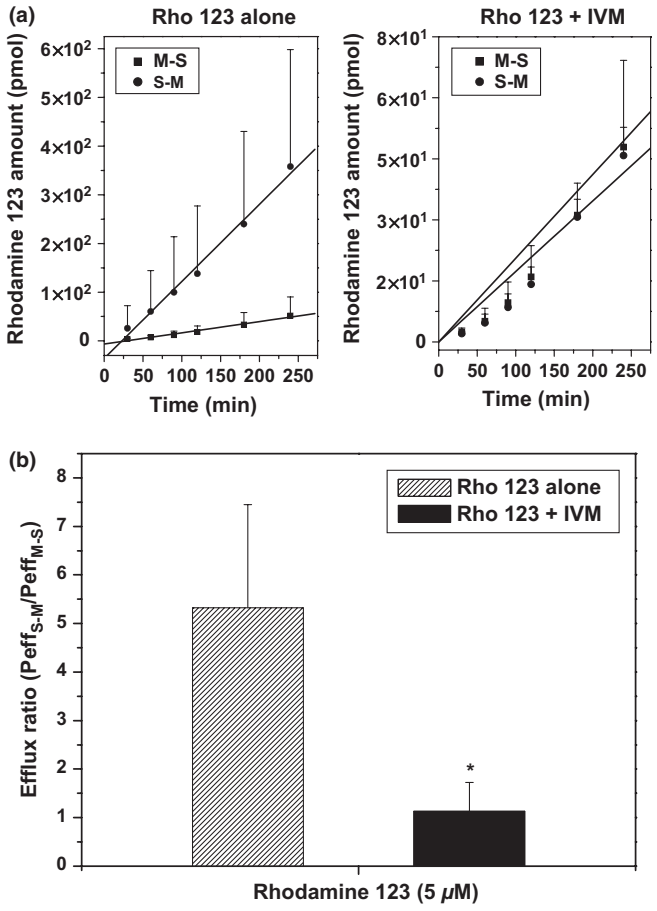

Fig. 4. (a) Rhodamine 123 (Rho 123) P-gp-mediated intestinal transport (mean ± SD) across the ileal segment of sheep intestine incubated either alone or with ivermectin (IVM) (10 μM). Passage was measured in the mucosal (S-M) or serosal (M-S) compartments of the Ussing chambers. The efflux ratio (P_{eff} S-M/P_{eff} M-S) (mean ± SD) for Rho 123 in sheep intestine incubated either alone or with IVM is shown in (b). Each value is an average of at least seven measurements. (*) Values are statistically different from those obtained after the incubation of Rho 123 alone at $P < 0.05$.

ABCB1 mRNA between liver and small intestine, as well as along the intestine, were observed. Quantitative PCR indicated that ABCB1 mRNA basal levels are significantly increased from oral to caudal, suggesting a gradient along the intestinal tract. This expression pattern in the small intestine of sheep was similar to that reported in humans and laboratory animals (Fojo *et al.*, 1987; Trezise *et al.*, 1992; Fricker *et al.*, 1996; Li *et al.*, 1999). However, differences in the P-gp mRNA profiles were described in horses where the highest P-gp gene expression was found in the duodenum and in the proximal portions of the jejunum (Tydén *et al.*, 2009). The physiological P-gp role at the intestinal mucosal epithelium greatly contributes not only to the direct excretion of transported xenobiotics into the intestinal lumen but also to reducing the uptake of orally administered drugs. In addition, animal and human studies corroborated that intestinal metabolic enzymes and efflux transporters, working coordinately as a protective mechanism, could be responsible for the poor bioavailability of a number of drugs (Benet, 2009). In veterinary medicine, the contribution of P-gp in the active biliary and intestinal secretion of drug compounds widely used in livestock, such as ivermectin and doramectin, has been demonstrated (Hennesy *et al.*, 2000; Lafont *et al.*, 2002).

The *in vivo* modulation of the P-gp activity by using specific inhibitors has been performed to study the contribution of cell transporters on the kinetic disposition of different antiparasitic drugs in the sheep (Molento *et al.*, 2004; Ballent *et al.*, 2007; Alvinerie *et al.*, 2008; Lifschitz *et al.*, 2010). An increased systemic availability of IVM has been obtained after their *in vivo* co-administration with different P-gp modulators (Ballent *et al.*, 2007; Alvinerie *et al.*, 2008). Additionally, an enhanced efficacy against resistant nematodes was observed after co-administration of IVM with a P-gp inhibitor in sheep (Lifschitz *et al.*, 2010). The induction of this efflux protein has been also recognized as a mechanism of drug–drug interactions. At intestinal level, P-gp induction mediated by several agents has been associated with reduced bioavailability of orally administered drugs (Lin, 2003; Machavaram *et al.*, 2006; Schwarz *et al.*, 2007). In rats, the pretreatment with the P-gp inducer phenobarbital resulted in decreased IVM plasma concentrations (Ballent *et al.*, 2010). Recently, it was reported that IVM can also induce P-gp expression and function through mRNA stabilization in murine hepatic cells (Ménez *et al.*, 2012). However, there is no available information on the *in vivo* relationship between P-gp induction and drug transport through the intestine of ruminant species. Dexamethasone is among the most widely prescribed drugs in large animal's therapy (Ferguson *et al.*, 2009). As a part of the same series of experiments performed in our laboratory, the effects of DEX on the expression and the metabolic activity of different CYP3A isoforms in sheep were assessed following the same induction protocol (Maté *et al.*, 2012). Considering the interplay and the overlap in the substrate selectivity between CYP3A and P-gp, the potential *in vivo* effect of repeated administrations of DEX on P-gp hepatic and intestinal expression was investigated. The selected dose (3 mg/kg) of DEX is that prescribed for the treatment of shock episodes in ruminant species (Escudero Pastor *et al.*, 2002). Under our experimental conditions, no significant differences regarding ABCB1 mRNA expression in the analyzed tissues of sheep were reached following the administration of DEX. Although an upregulation mediated by DEX on P-gp gene expression in the liver and intestine of rats was demonstrated (Yokogawa *et al.*, 2002), some studies have reported a reduction in the P-gp mRNA expression levels in different organs, including gastrointestinal tissues, after administration of DEX using different dosage regimens in laboratory animals (Salphati & Benet, 1998; Sérée *et al.*, 1998; Micuda *et al.*, 2007). In the current work, the amount of ABCB1 mRNA along the small intestine was reduced in a range of 30%–60% compared with the untreated control animals. Multiple factors have been proposed to explain these discrepancies. Among them, tissue-specific induction (Matheny *et al.*, 2004), origin of the cell culture used, animal species (Quattrochi & Guzelian, 2001; Narang

et al., 2008), and DEX dosage (Cantiello *et al.*, 2009) have been associated with the variability in the observed response to DEX treatments. It is also recognized that efflux proteins are subjected to transcriptional as well as post-transcriptional regulation. At the transcriptional level, hepatic and intestinal metabolizing enzymes and transporter proteins are regulated by a variety of transcription factors (TFs) activated by ligands in response to exogenous and endogenous activators. Among these nuclear receptors, the pregnane X receptor (PXR) and the constitutive androstane receptor (CAR) are the best-studied TFs implicated in the expression of P-gp induced by xenobiotics (Kast *et al.*, 2002; Maglich *et al.*, 2002). PXR and CAR transcription and translation seem to be enhanced by different glucocorticoids including DEX (Pascussi *et al.*, 2000). To further investigate the potential role of these TFs on the regulation of P-gp expression, PXR mRNA (NM_001103226) and CAR mRNA (NM_001079768) levels were also measured in the liver of sheep that received the same treatment with DEX. In agreement with our results on ABCB1 mRNA expression, neither PXR nor CAR was modified under the current induction regimen (L. Maté, personal communication).

Experimental assays correlating *in vitro–in vivo* information are relevant to understand the relationship between expression and activity of transporter proteins such as P-gp. The use of cell cultures that overexpress efflux proteins to measure P-gp activity does not correlate well across species. As the functional architecture of intestinal mucosa is preserved, this study demonstrates that the Ussing chamber technique can be used as an *ex vivo* method to measure the intestinal efflux mediated by P-gp in ruminants. In the present work, functional studies in diffusion chambers were also performed in the ileum of control and DEX-treated animals. The Ussing chamber technique was correctly validated to characterize the active transport of Rho 123, a fluorescent P-gp substrate, which was used as a marker of the P-gp efflux activity in the ileum of experimental animals. To confirm the involvement of P-gp on intestinal efflux of Rho 123 in the sheep, inhibition studies using IVM as a P-gp inhibitor were performed. The intestinal efflux of Rho 123 was significantly reduced by the presence of IVM. Although the P_{eff} from serosal to mucosal tended to be higher in the intestine of DEX-treated animals compared with the controls, no statistical differences were reached, being in agreement with the expression studies. Consistently, in previously reported *in vitro* studies neither Rho 123 accumulation (Hamilton *et al.*, 2001) nor Rho 123 biliary excretion (Annaert *et al.*, 2001) was affected by the presence of DEX. In conclusion, the current work characterized for the first time the expression of P-gp along the gastrointestinal tract of sheep. Besides, this study describes an *ex vivo* assay to characterize sheep P-gp intestinal activity using intact tissue. As there is no available information on the *in vivo* effect of potential P-gp inducers in ruminant species, a better understanding of the factors regulating P-gp expression and activity could contribute to elucidate clinical implications for drug therapy in livestock animals.

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REFERENCES

Alvinerie, M., Dupuy, J., Kiki-Mvouaka, S., Sutra, J.F. & Lespine, A. (2008) Ketoconazole increases the plasma levels of ivermectin in sheep. *Veterinary Parasitology*, **157**, 117–122.

Annaert, P., Turncliff, R., Booth, C., Thakker, D. & Brouwer, K. (2001) P-glycoprotein-mediated in vitro biliary excretion in sandwich-cultured rat hepatocytes. *Drug Metabolism and Disposition*, **29**, 1277–1283.

Ballent, M., Lifschitz, A., Virkel, G., Sallovitz, J.M. & Lanusse, C. (2007) Involvement of P-glycoprotein on ivermectin kinetic behaviour in sheep: itraconazole-mediated changes on gastrointestinal disposition. *Journal of Veterinary Pharmacology and Therapeutics*, **30**, 242–248.

Ballent, M., Lifschitz, A., Virkel, G., Maté, L. & Lanusse, C. (2010) Pretreatment with the inducers rifampicin and phenobarbital alters ivermectin gastrointestinal disposition. *Journal of Veterinary Pharmacology and Therapeutics*, **33**, 252–259.

Benet, L.Z. (2009) The drug transporter-metabolism alliance: uncovering and defining the interplay. *Molecular Pharmacology*, **6**, 1631–1643.

Cantiello, M., Giantin, M., Carletti, M., Lopparelli, R.M., Capolongo, F., Lasserre, F., Bollo, E., Nebbia, C., Martin, P.G., Pineau, T. & Dacasto, M. (2009) Effects of dexamethasone, administered for growth promoting purposes, upon the hepatic cytochrome P4503A expression in the veal calf. *Biochemical Pharmacology*, **77**, 386–451.

Escudero Pastor, E., Cáceres Rodriguez, C., Serrano Caballero, J. & Ponferrada Abrisqueta, C. (2002) Adrenal hormones and related drugs. In *Veterinary Pharmacology and Therapeutics*. Eds. Botana, L., Landoni, F. & Martín-Jiménez, T., pp. 391–407. McGraw Hill, Interamericana, Madrid.

Ferguson, D., Dirikolu, L. & Hoenig, M. (2009) Glucocorticoids, mineralocorticoids, and adrenolytic drugs. In *Veterinary Pharmacology and Therapeutics*, 9th edn. Eds Riviere, J., Papich, M., pp. 771–802. Wiley-Blackwell, Ames.

Fojo, A.T., Ueda, K., Slamon, D.G., Poplack, D.G., Gottesman, M.M. & Pastan, I. (1987) Expression of the multidrug-resistance gene in human tumors and tissues. *Proceedings of the National Academy of Sciences USA*, **84**, 265–269.

Fricker, G., Drewe, J., Huwyler, J., Gutmann, H. & Beglinger, C. (1996) Relevance of P-glycoprotein for the enteral absorption of cyclosporin A: in vitro-in vivo correlation. *British Journal of Pharmacology*, **118**, 1841–1847.

Hamilton, K., Yazdanian, M. & Audus, K. (2001) Modulation of P-glycoprotein activity in Calu-3 cells using steroids and β-ligands. *International Journal of Pharmaceutics*, **228**, 171–179.

Hennesy, D.R., Page, S.W. & Gottschall, J. (2000) Behaviour of doramectin in the gastrointestinal tract, its secretion in bile and pharmacokinetic disposition in the peripheral circulation after oral and intravenous administration to sheep. *Journal of Veterinary Pharmacology and Therapeutics*, **23**, 203–213.

Huang, L., Wring, S.A., Woolley, J.L., Brouwer, K.R., Serabjit-Singh, C. & Polli, J.W. (2001) Induction of P-glycoprotein and cytochrome P450 3A by HIV protease inhibitors. *Drug Metabolism and Disposition*, **29**, 754–760.

Kast, H.R., Goodwin, B., Tarr, P.T., Jones, S.A., Anisfeld, A.M., Stoltz, C.M., Tontonoz, P., Kliewer, S., Willson, T.M. & Edwards, P.A. (2002) Regulation of multidrug resistance-associated protein 2 (ABCC2) by the nuclear receptors pregnane X receptor, farnesoid X-activated receptor, and constitutive androstane receptor. *Journal of Biological Chemistry*, **277**, 2908–2915.

Laffont, C.M., Toutain, P.L., Alvienrie, M. & Bousquet-Mélou, A. (2002) Intestinal secretion is a major route of ivermectin elimination in the rat. *Drug Metabolism and Disposition*, **30**, 626–630.

Li, M., Hurren, R., Zastawny, R.L., Ling, V. & Buick, R.N. (1999) Regulation and expression of multidrug resistance (MDR) transcripts in the intestinal epithelium. *British Journal of Cancer*, **80**, 1123–1131.

Lifschitz, A., Entrocasso, C., Alvarez, L., Lloberas, M., Ballent, M., Manazza, G., Virkel, G., Borda, B. & Lanusse, C. (2010) Interference with P-glycoprotein improves ivermectin activity against adult resistant nematodes in sheep. *Veterinary Parasitology*, **172**, 291–298.

Lin, L.H. (2003) Drug-drug interaction mediated by inhibition and induction of P-glycoprotein. *Advanced Drug Delivery Reviews*, **55**, 53–81.

Livak, K. & Schmittgen, T. (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods*, **25**, 402–408.

Machavaram, K.K., Gundu, J. & Yamsani, M.R. (2006) Effect of ketoconazole and rifampicin on the pharmacokinetics of ranitidine in healthy human volunteers: a possible role of P-glycoprotein. *Drug Metabolism and Drug Interactions*, **22**, 47–65.

Maglich, J.M., Stoltz, C.M., Goodwin, B., Hawkins-Brown, D., Moore, J. T. & Kliewer, S.A. (2002) Nuclear pregnane x receptor and constitutive androstane receptor regulate overlapping but distinct sets of genes involved in xenobiotic detoxification. *Molecular Pharmacology*, **62**, 638–646.

Martin, P., Riley, R., Back, D.J. & Owen, A. (2008) Comparison of the induction profile for drug disposition proteins by typical nuclear receptor activators in human hepatic and intestinal cells. *British Journal of Pharmacology*, **153**, 805–819.

Maté, L., Lifschitz, A., Sallovitz, J., Ballent, M., Muscher, A.S., Wilkens, M.R., Schroder, B., Lanusse, C. & Virkel, G. (2012) Cytochrome P450 3A expression and function in liver and intestinal mucosa from dexamethasone-treated sheep. *Journal of Veterinary Pharmacology and Therapeutics*, **35**, 319–328.

Matheny, C.J., Ali, R.Y., Yang, X. & Pollack, G.M. (2004) Effect of prototypical inducing agents on P-glycoprotein and CYP3A expression in mouse tissues. *Drug Metabolism and Disposition*, **32**, 1008–1014.

Mayer, U., Wagenaar, E., Dorobek, B., Beijnen, J., Borst, P. & Schinkel, A. (1997) Full blockade of intestinal P-glycoprotein and extensive inhibition of blood-brain barrier P-glycoprotein by oral treatment of mice with PSC833. *Journal of Clinical Investigations*, **100**, 2430–2436.

Ménez, C., Mselli-Lakhal, L., Foucaud-Vignault, M., Balaguer, P., Alvinerie, M. & Lespine, A. (2012) Ivermectin induces P-glycoprotein expression and function through mRNA stabilization in murine hepatocyte cell line. *Biochemical Pharmacology*, **83**, 269–278.

Micuda, S., Fuksa, L., Mundlova, L., Osterreicher, J., Mokry, J., Cermanova, J., Brcakova, E., Staud, F., Pokorna, P. & Martinkova, J. (2007) Morphological and functional changes in P-glycoprotein during dexamethasone-induced hepatomegaly. *Clinical and Experimental Pharmacology and Physiology*, **34**, 296–303.

Molento, M.B., Lifschitz, A., Sallovitz, J., Lanusse, C. & Prichard, R. (2004) Influence of verapamil on the pharmacokinetics of the antiparasitic drugs ivermectin and moxidectin in sheep. *Parasitology Research*, **92**, 121–127.

Narang, V.S., Fraga, C., Kumar, N., Shen, J., Throm, S., Stewart, C.F. & Waters, C.M. (2008) Dexamethasone increases expression and activity of multidrug resistance transporters at the rat blood-brain barrier. *American Journal of Physiology- Cell Physiology*, **295**, 440–450.

Pascussi, J., Gerbal-Chaloin, S., Fabre, F., Maurel, P. & Vilarem, M. (2000) Dexamethasone enhances constitutive androstane receptor expression in human hepatocytes: consequences on cytochrome P450 gene regulation. *Molecular Pharmacology*, **58**, 1441–1450.

Quattrochi, L.C. & Guzelian, P.S. (2001) Cyp3A regulation: from pharmacology to nuclear receptors. *Drug Metabolism and Disposition*, **29**, 615–622.

Salphati, L. & Benet, L.Z. (1998) Modulation of P-glycoprotein expression by cytochrome P450 3A inducers in male and female rat livers. *Biochemical Pharmacology*, **55**, 387–395.

Schinkel, A.H., Wagenaar, E., Mol, C.A. & Van Deemter, L. (1996) P-glycoprotein in the blood-brain barrier of mice influences the brain penetration and pharmacological activity of many drugs. *Journal of Clinical Investigations*, **97**, 2517–2524.

Schuetz, E.G., Beck, W.T. & Schuetz, J.D. (1996) Modulators and substrates of P-glycoprotein and cytochrome P4503A coordinately up-regulate these proteins in human colon carcinoma cells. *Molecular Pharmacology*, **49**, 311–318.

Schwarz, U.I., Hanso, H., Oertel, R., Miehlke, S., Kuhlisch, E., Glaeser, H., Hitzi, M., Dresser, G.K., Kim, R.B. & Kirch, W. (2007) Induction of intestinal P-glycoprotein by St John′s wort reduces the bioavailability of talinolol. *Clinical Pharmacology and Therapeutics*, **81**, 669–678.

Sérée, E., Villard, P.H., Hevér, A., Guigal, N., Puyoou, F., Charvet, B., Point-Scomma, H., Lechevalier, E., Lacarelle, B. & Barra, Y. (1998) Modulation of MDR1 and CYP3A expression by dexamethasone: evidence for an inverse regulation in adrenals. *Biochemical and Biophysical Research Communications*, **18**, 392–395.

Song, S., Suzuki, H., Kawai, R. & Sugiyama, Y. (1999) Effect of PSC 833, a P-glycoprotein modulator, on the disposition of vincristine and digoxin in rats. *Drug Metabolism and Disposition*, **27**, 689–694.

Thiebaut, F., Tsuruo, T., Hamada, H., Gottesman, M.M., Pastan, I. & Willingham, M.C. (1987) Cellular localization of the multidrug-resistance gene product P-glycoprotein in normal human tissues. *Proceedings of the National Academy of Sciences USA*, **84**, 7735–7738.

Trezise, A.E., Romano, P.R., Gill, D.R., Hyde, S.C., Sepúlveda, F.V., Buchwald, M. & Higgins, C.F. (1992) The multidrug resistance and cystic fibrosis genes have complementary patterns of epithelial expression. *The EMBO Journal*, **11**, 4291–4303.

Tydén, E., Tallkvist, J., Tjälve, H. & Larsson, P. (2009) P-glycoprotein in intestines, liver, kidney and lymphocytes in horse. *Journal of Veterinary Pharmacology and Therapeutics*, **32**, 167–176.

Yokogawa, K., Shimada, T., Higashi, Y., Itoh, Y., Masue, T., Ishizaki, J., Asahi, M. & Miyamoto, K. (2002) Modulation of mdr1a and CYP3A gene expression in the intestine and liver as possible cause of changes in the cyclosporine A disposition kinetics by dexamethasone. *Biochemical Pharmacology*, **15**, 777–783.

Yumoto, R., Murakami, T., Sanemasa, M., Nasu, R., Nagai, J. & Takano, M. (2001) Pharmacokinetic interaction of cytochrome P450 3A-related compounds with rhodamine 123, a P-glycoprotein substrate, in rats pretreated with dexamethasone. *Drug Metabolism and Disposition*, **29**, 145–151.

Zahner, D., Alber, J. & Petzinger, E. (2010) Cloning an heterologous expression of the ovine (ovis aries) P-glycoprotein (Mdr1) in Madin-Darby canine kidney (MDCK) cells. *Journal of Veterinary Pharmacology and Therapeutics*, **33**, 304–311.