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Effects of triclabendazole on secretion of danofloxacin and moxidectin into the milk of sheep: Role of triclabendazole metabolites as inhibitors of the ruminant ABCG2 transporter

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ABSTRACT

ATP-binding cassette transporter G2/breast cancer resistance protein (ABCG2/BCRP) mediates drug–drug interactions that affect the secretion of drugs into milk. The aims of this study were: (1) to determine whether the major plasma metabolites of the flukicide triclabendazole (TCBZ), triclabendazole sulfoxide (TCBZSO) and triclabendazole sulfone ($TCBZSO_2$), inhibit ovine and bovine ABCG2 and its Y581S variant in vitro, and (2) to examine whether coadministration of TCBZ with the ABCG2 substrates danofloxacin (a fluoroquinolone) and moxidectin (a milbemycin) affects the secretion of these drugs into the milk of sheep.

TCBZSO and $TCBZSO_2$ inhibited ruminant ABCG2 in vitro by reversing the reduced mitoxantrone accumulation and reducing basal to apical transport of nitrofurantoin in cells transduced with bovine variants (S581 and Y581) and the ovine variant of ABCG2. Coadministration of TCBZ with moxidectin or danofloxacin to sheep resulted in significantly reduced levels of moxidectin, but not danofloxacin, in the milk of TCBZ-treated sheep compared to sheep administered moxidectin or danofloxacin alone. The milk area under concentration time curve (AUC 0–48 h) was 2.99 ± 1.41 μg h/mL in the group treated with TCBZ and moxidectin, and 7.75 ± 3.58 μg h/mL in the group treated with moxidectin alone. The AUC (0–48 h) milk/plasma ratio was 37% lower in the group treated with TCBZ and moxidectin (7.34 ± 1.51) than in the group treated with moxidectin alone (11.68 ± 3.61). TCBZ metabolites appear to inhibit ruminant ABCG2 and affect the secretion of ABCG2 substrates into milk of sheep.

Introduction

ATP-binding cassette (ABC) transporters are major determinants of the efflux of a wide variety of drugs, protecting against the toxicity of xenobiotics and influencing pharmacokinetics and pharmacodynamics. ABC efflux proteins in parasites are important in anthelmintic resistance (Lespine et al., 2012). Milbemycins (such as moxidectin), benzimidazoles (such as albendazole sulfoxide and oxfendazole) and fluoroquinolones (such as enrofloxacin and danofloxacin) are substrates of the ABC transporter ABCG2 (Perez et al., 2009a; Real et al., 2011a; Mealey, 2012). Induced expression of ABCG2 during lactation is related to its role in active drug secretion into milk (Jonker et al., 2005).

Multiple drugs are often administered concomitantly in veterinary therapy, which may affect their kinetics and pharmacological activity. Drug–drug interactions mediated by ABC transporters include increased plasma availability and potentiation of the macrocyclic lactones ivermectin and moxidectin after coadministration of the anthelmintic triclabendazole (TCBZ) or inhibitors of the ABC transporter P-glycoprotein (Lifschitz et al., 2009; McKellar and Gokbulut, 2012). In Assaf sheep, secretion of danofloxacin into milk is reduced on coadministration with the macrocyclic lactone ivermectin (Real et al., 2011a) or by supplementation with a soy-enriched diet (Perez et al., 2013).

Genetic variations in ABCG2 should also be considered, since the bovine ABCG2 Y581S single nucleotide polymorphism (SNP) is associated with increased secretion of danofloxacin into milk (Otero et al., 2013), differential inhibition of the macrocyclic lactone ivermectin in vitro and increased transepithelial transport of antibiotics in vitro (Real et al., 2011b).

Triclabendazole (TCBZ) is a halogenated benzimidazole thiol derivative used for the treatment of liver fluke (*Fasciola* spp.) infestation (Fairweather, 2009). The parent drug TCBZ is not detected in

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plasma or milk after its oral administration in sheep, because it is rapidly metabolised into triclabendazole sulfoxide (TCBZSO) and triclabendazole sulfone ($TCBZSO_2$) (Hennessy et al., 1987; Imperiale et al., 2011). Triclabendazole metabolites are good inhibitors of the murine and human variants of ABCG2 (Barrera et al., 2012). However, their relevance for drug–drug interactions in ruminants remains to be established.

The aims of the present study were (1) to determine whether the major plasma TCBZ metabolites (TCBZSO and $TCBZSO_2$) in vitro inhibit the two bovine and the ovine ABCG2 variants, and (2) to assess the relevance of TCBZ metabolites in in vivo drug–drug interactions by studying the effect of TCBZ coadministration on the disposition of the ABCG2 substrates moxidectin (Perez et al., 2009a) and danofloxacin (Real et al., 2011a) in the plasma and milk of sheep.

Materials and methods

Cell cultures

Madin-Darby canine kidney epithelial cell (MDCKII) parental cells were provided by Dr A.H. Schinkel, Netherlands Cancer Institute, Amsterdam, The Netherlands. MDCKII cells were stably transduced with both bovine variants (S581 and Y581) and the ovine variant of ABCG2 (Real et al., 2011b). Culture conditions were as described previously (Jonker et al., 2000; Pavek et al., 2005).

Accumulation assays

In vitro accumulation assays were carried out as described previously (Pavek et al., 2005). Mitoxantrone (MXR, 10 μM; Sigma–Aldrich) was used as a fluorescent substrate and the compounds tested were used as inhibitors. Relative cellular accumulation of MXR was determined by flow cytometry using a CYAN cytometer (Beckman Coulter) from histogram plots using the median of fluorescence (MF). ABCG2 inhibition increases the accumulation of MXR in ABCG2-transduced cells and thus increases MF. Inhibitory potencies were calculated as described previously (Pavek et al., 2005): Inhibitory potency = (MF with tested compound − MF without inhibitor)/(MF with Ko143 − MF without inhibitor) × 100%. Ko143 (1 μM; Tocris) has been described previously as a potent and specific ABCG2 inhibitor (Allen et al., 2002).

Transport studies

Transport assays using Transwell plates were carried out as described previously (Merino et al., 2010; Real et al., 2011b), with minor modifications. Cells were seeded on microporous polycarbonate membrane filters (3.0 μm pore size, 24 mm diameter; Transwell 3414; Costar) at a density of 1.0×10^6 cells per well and grown for 3 days. Transepithelial resistance, as measured in each well using a Millicell ERS ohmmeter (Millipore), was used to check the integrity of the monolayer. The appearance of nitrofurantoin (Sigma–Aldrich) in the acceptor compartment was presented as the fraction of total nitrofurantoin added to the donor compartment at the beginning of the experiment.

Pharmacokinetic studies

The experiments were performed on the Experimental Farm of the University of Leon, Spain, and were approved by the Research Committee for Animal Use of the University of Leon (approval number 13-2011, date of approval 7 November 2011).

Twenty-four lactating Assaf sheep (3–4 months in lactation), aged 2–3 years and weighing 70–75 kg, were used in this study. The animals were parasite-free and drinking water was available ad libitum. The experimental design was performed with animals divided into four groups: (1) the first group ($n = 6$) received a single dose of 1.25 mg/kg danofloxacin (Advocin 2.5%, Pfizer) IM; (2) the second group ($n = 6$) was injected with 1.25 mg/kg danofloxacin (Advocin 2.5%) IM coadministered with 1 mg/kg TCBZ (Sequoia Research Products) IV; (3) the third group ($n = 6$) received a single dose of 0.2 mg/kg moxidectin (Cydectin 1%, Fort Dodge) SC; (4) and the fourth group ($n = 5$) was injected with 0.2 mg/kg moxidectin (Cydectin 1%) SC coadministered with 2 mg/kg TCBZ (Fasinex 10%) PO. The dosages and routes of administration of TCBZ were chosen in an attempt to achieve similar plasma profiles of the TCBZ metabolites and the coadministered drugs and to avoid concentrations in milk >0.20 μg/mL (close to the maximum limits of TCBZ residues in target tissues) (EMEA, 2006).

Blood samples were collected from the jugular vein and milk samples were collected after complete milking of the gland before each treatment, at 0.25, 0.5, 1, 2, 4, 6, 8, 10, 12 and 24 h after danofloxacin administration (groups 1 and 2), and at 1, 4, 8, 12, 24, 36, 48, 72, 96, 120, 192 and 360 h after moxidectin administration (groups 3 and 4). Plasma was separated by centrifugation at 1200 *g* for 15 min and plasma and milk samples were stored at −20 °C until analysis.

High performance liquid chromatography

High performance liquid chromatography (HPLC) was used to determine concentrations of danofloxacin in plasma and milk in experimental groups 1 and 2, and concentrations of moxidectin in plasma and milk in experimental groups 3 and 4. Levels of the major plasma and milk TCBZ metabolites were analysed in the TCBZ-treated groups (groups 2 and 4) by HPLC.

Conditions for HPLC of nitrofurantoin were modified in accordance with Merino et al. (2005). The mobile phase consisted of 25 mM potassium phosphate buffer (pH 3):acetonitrile (75:25), the flow rate of the mobile phase was set to 1.2 mL/min and ultraviolet (UV) absorbance was measured at 366 nm. Standard samples for calibration curves were prepared at concentrations of 0.04–5 μg/mL, with coefficients of correlation >0.99. The limit of quantification (LOQ) was 40 ng/mL.

Conditions for HPLC analysis of danofloxacin were modified in accordance with Perez et al. (2013). Marbofloxacin (Sigma–Aldrich) was used as an internal standard and samples were extracted with chloroform. The mobile phase consisted of 25 mM orthophosphoric acid (pH 3.0):acetonitrile (75:25) at a flow rate of 1.5 mL/min. Fluorescence was detected at 338 nm (excitation) and 425 nm (emission). Standard samples of danofloxacin (Sigma–Aldrich) for calibration curves were prepared at concentrations of 0.01–0.64 μg/mL for plasma and 0.16–10 μg/mL for milk, with coefficients of correlation >0.99. The extraction recovery levels for concentrations in the standard curve were 85% for plasma and 86% for milk. LOQs were 8.2 ng/mL for plasma and 100 ng/mL for milk.

Conditions for HPLC analysis of moxidectin were modified in accordance with Prieto et al. (2003). Ivermectin (Sigma–Aldrich) was used as an internal standard and samples were extracted with methanol and derivatised. The mobile phase consisted of acetonitrile:methanol:water with 0.2% acetic acid (45%:50%:5%, V/V/V) at a flow rate of 1.8 mL/min. Fluorescence was detected at 365 nm (excitation) and 475 nm (emission). Standard samples of moxidectin (Sigma–Aldrich) for calibration curves were prepared at concentrations of 0.001–0.06 μg/mL for plasma and 0.004–0.36 μg/mL for milk, with coefficients of correlation >0.99. The extraction recovery levels for concentrations in the standard curve were 82% for plasma and 85% for milk. LOQs were 1 ng/mL for plasma and 3 ng/mL for milk.

Conditions for HPLC analysis of TCBZ metabolites were modified in accordance with Imperiale et al. (2011). Oxibendazole (Sigma–Aldrich) was used as an internal standard and samples were extracted with acetonitrile. The mobile phase consisted of 25 mM ammonium acetate (pH: 6.6):acetonitrile (48:52) at a flow rate of 1.2 mL/min and UV absorbance was measured at 300 nm. Standard samples of TCBZSO (LGC Standards) and $TCBSO_2$ (LGC Standards) for calibration curves were prepared at concentrations of 0.08–10 μg/mL for plasma and 0.04–0.625 μg/mL for milk, with coefficients of correlation >0.99. The extraction recovery levels for concentrations in the standard curve for TCBZSO were 72% for plasma and 71% for milk, and for $TCBZSO_2$ were 77% for plasma and 76% for milk. LOQs for TCBZSO were 52.8 ng/mL for plasma and 24.1 ng/mL for milk. LOQs for $TCBZSO_2$ were 9.6 ng/mL for plasma and 21.6 ng/mL for milk.

Statistical analysis

Results are reported as the mean ± standard deviation (SD). Statistical analysis for significant differences was performed using the two-tailed Student's *t* test. A probability of $P < 0.05$ was considered to be statistically significant.

Results

Inhibitory potency of the two TCBZ metabolites in mitoxantrone accumulation assays

To demonstrate the potential inhibitory effect of major plasma metabolites of TCBZ (TCBZSO and $TCBZSO_2$) on ruminant ABCG2, the ability of these compounds to reverse the reduced mitoxantrone accumulation in cells transduced with both bovine variants (S581 and Y581) and the ovine variant of ABCG2 was tested in flow cytometry experiments. The presence of either TCBZ metabolite inhibited all the ruminant ABCG2 variants, increasing the accumulation of mitoxantrone in ABCG2-transduced cells in a concentration-dependent manner. The highest inhibitory potency (60–70%) appeared at 25 μM for the bovine Y581 variant (Figs. 1A and B).