Short communication

Ivermectin reduces secretion of meloxicam into milk by inhibition of ABCG2 transporter in sheep

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Abstract

The ATP-binding cassette transporter G2 (ABCG2) is an efflux protein involved in the bioavailability and secretion into milk of several compounds including anti-inflammatory drugs. The aim of this work was to determine the effect in sheep of an ABCG2 inhibitor, such as the macrocyclic lactone ivermectin, on the secretion into milk of meloxicam, a non-steroidal anti-inflammatory drug widely used in veterinary medicine, and recently reported as an ABCG2 substrate in mice. In vitro meloxicam transport assays in ovine ABCG2-transduced cells have shown that meloxicam is a substrate of ovine ABCG2 and that ivermectin is an efficient inhibitor of in vitro transport of meloxicam mediated by ovine ABCG2. In addition, the role of ovine ABCG2 in secretion into milk of meloxicam was corroborated using Assaf lactating sheep coadministered with ivermectin. Animals were administered subcutaneously with meloxicam (0.5 mg/kg) with or without ivermectin (0.2 mg/kg). A significantly lower concentration of meloxicam in milk was detected when ivermectin was coadministered, revealing a major role of ABCG2 in the secretion into milk of meloxicam and a relevant drug-drug interaction affecting this process. These results will contribute to the understanding of the potential factors that modulate the transfer of anti-inflammatory drugs into milk, opening their potential use in lactating ruminants, and the effect of drug coadministration on the presence of milk residues of these compounds.

Keywords: ABCG2; Ivermectin; Meloxicam; Milk; Sheep.

Non-steroidal anti-inflammatory drugs (NSAIDs) are widely used for their analgesic, anti-inflammatory and antipyretic properties in human and veterinary medicine (Lees et al., 2004). Meloxicam is an NSAID with high therapeutic potential in ruminants for pain (Colditz et al., 2019). Great benefits of the use of meloxicam in typical dairy cattle diseases have been described. In fact, treatments with meloxicam reduce pain, edema, temperature and number of somatic cells count caused by mastitis (McDougall et al., 2009; Fitzpatrick et al., 2013), which implies economic benefits for farmers (van Soest et al., 2018). However, its use in lactating cattle is reduced due to its high withdrawal period in milk (European Medicines Agency, 2019).

The ATP-binding cassette transporter G2 (ABCG2) is one of the main factors involved in the active secretion of many compounds into milk, including veterinary drugs (Mealey, 2012; Mahnke et al., 2016; Garcia-Lino et al., 2019; Imperiale and Lanusse, 2021; Blanco-Paniagua et al., 2022) and also specifically anti-inflammatory drugs (García-Mateos et al., 2019). Interest is focused on gaining information about potential mechanisms to reduce withdrawal periods and about factors influencing the appearance of drug residues in milk. For instance, drug-drug interactions leading to the inhibition of ABCG2 result in variation in drug secretion into milk (Real et al., 2011; Barrera et al., 2013).

Recently, ABCG2 has been identified as an important determinant of the secretion into milk of meloxicam using Abcg2-knockout mice (Garcia-Lino et al., 2020). However, whether this finding can be extrapolated to the secretion into milk of meloxicam in ruminants is unknown. In this study, therefore, the effect of a known ABCG2 inhibitor, such as the macrocyclic lactone ivermectin (Merino et al., 2009), on the secretion of meloxicam into milk was studied in sheep.

Beforehand, in vitro ovine ABCG2-mediated transport of meloxicam and the role of ivermectin as an inhibitor of this process were assessed in vectorial transport assays using Transwell plates with MDCKII cells transduced with ovine variant of ABCG2, as previously described (González-Lobato et al., 2014). Parental Madin-Darby Canine Kidney (MDCKII) cells and MDCKII cells transduced with ovine variant of ABCG2 were seeded on microporous polycarbonate membrane filters at a density of 1.0 x 10^6 cells per well. To check the tightness of the monolayer, transepithelial resistance was measured in each well using a Millicell ERS ohmmeter (Millipore). The presence of meloxicam (Sigma-Aldrich) in the acceptor compartment was presented as the fraction of total meloxicam added to the donor compartment at the beginning of the experiment. Active transport across MDCKII monolayers was expressed by the relative transport ratio (R), defined as the apically directed transport percentage divided by the basolaterally directed translocation percentage, after 4 h. Samples were analyzed by HPLC as described previously (Garcia-Lino et al., 2020). Standard samples in appropriate drug-free matrix were prepared and coefficients of correlation were > 0.99. The limit of quantification (LOQ) was 0.01 µg/mL. Statistical analysis for significant differences was performed using the Student's t-test (normal variables) and the Mann-Whitney U test (not normally distributed variables). All analyses were carried out on the assumed significance level of $p \leq 0.05$ using SPSS Statistics software (v. 24.0; IBM, Armonk, New York, NY, USA).

Table 1 shows the results obtained in the meloxicam transport assay using ivermectin at 10 µM as ABCG2 inhibitor. In parental cells, apical to basal directed translocation was equal to basal to apical translocation of meloxicam (Relative transport ratio close to 1). However, in the ovine ABCG2-transduced cells, as has already been reported for murine Abcg2 (Garcia-Lino et al., 2020), apical to basal directed translocation was highly decreased and basal to apical directed translocation was

increased compared with the MDCKII parental cell line. Subsequently, the relative efflux transport ratio at 4 h was significantly higher in the ovine ABCG2-transduced cells (24.85 ± 4.6 vs 1.06 ± 0.08, $p \leq 0.05$), indicating that meloxicam is an in vitro substrate for ovine ABCG2-transduced cells. When ivermectin at 10 µM was added, a reduction of 75% in the relative transport ratio of meloxicam was reported in the cells transduced with ovine ABCG2 (24.85 ± 4.62 vs 6.31 ± 1.37, $p \leq 0.05$). No differences in the transport ratio of meloxicam were observed comparing parental cells with or without ivermectin. These results show that ivermectin inhibits meloxicam transport mediated by ovine ABCG2, as shown previously for other substrates (Merino et al., 2009; Real et al., 2011).

Therefore, to check for possible in vivo interactions, studies with sheep were conducted according to institutional guidelines complying with European legislation (2010/63/EU), and approved by the Animal Care and Use Committee of the University of León and Junta de Castilla y León ULE_008_2016 (09/06/2016). Lactating Assaf sheep (3–4 months in lactation) and weighing 70 to 85 kg were divided into 2 groups, and received a subcutaneous injection of 0.5 mg/kg of Metacan® (20 mg/mL) with or without the co-administration of a subcutaneous dose of ivermectin (Ivomec®) (0.2 mg/kg). The animals were parasite-free and drinking water was available ad libitum. The normal milking routine for all the animals involved milk being taken twice each day. Blood samples were collected from the jugular vein and milk samples were collected after completing milking of the gland before each treatment at 0.5, 1, 2, 4, 6, 8, 10, 12, 24, 36, 48 and 72 h after meloxicam administration. Plasma was separated by centrifugation at 3000 x g for 15 min. The conditions for the HPLC analysis have been described previously (Garcia-Lino et al., 2020). Standard samples in appropriate drug-free matrix were prepared and coefficients of correlation were > 0.99. The extraction recovery levels

for concentration in the standard curve were 88% for plasma and 90 % for milk samples. The LOQs were 0.02 µg/mL for plasma and 0.02 µg/mL for milk.

No relevant differences in plasma levels of meloxicam were found between both groups of animals (Fig. 1A). Meloxicam plasma levels for both groups were similar to those reported previously in sheep (Shukla et al., 2007; Woodland et al., 2019). The absence of differences in plasma concentration is reflected in the pharmacokinetic parameters (Table 2). Despite the lack of differences in plasma, a lower milk concentration of meloxicam was found in the animals coadministered with ivermectin at 12 and 30 h (Fig. 1B). The values of the area under concentration-time curve ($AUC_{(0-\infty)}$) for milk and the AUC milk-to-plasma ratio were reduced by more than 40% in ivermectin coadministered animals compared with control animals (Table 2). Although ivermectin interacts with other ABC transporters, such as P-glycoprotein (Lespine et al., 2009), the effect of ivermectin on meloxicam secretion into sheep milk can be attributed to ABCG2-mediated interaction since no other ABC transporters are substantially expressed or induced in lactating mammary gland (Van Herwaarden and Schinkel, 2006). This kind of drug-drug interaction mediated by the ABCG2 transporter has been observed previously with the co-administration of ivermectin and other ABCG2 substrates, such as the antimicrobial danofloxacin, in sheep (Real et al., 2011). The present data show that secretion into milk of meloxicam can be modulated by ivermectin, producing drug-drug interaction, but also probably by other compounds that interact with the ABCG2 transporter, as other drugs or molecules present in the diet such as flavonoids (Pulido et al., 2006; Otero et al., 2018), with consequences regarding the amount of milk residues.

In conclusion, the major role of ABCG2 in the secretion of meloxicam into ovine milk and the effect of drug-drug interactions in this process using the macrocyclic lactone ivermectin as inhibitor of the transporter are demonstrated. These results will contribute

to the understanding of the factors that influence the transfer of anti-inflammatory drugs into ruminant milk.

Declaration of interest: none

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Figure legends

Figure 1. Concentration in plasma (A) and milk (B) vs. time curves for meloxicam obtained from lactating Assaf sheep treated with a single dose of meloxicam (Metacam®) at 0.5 mg/kg (s.c.) and co-administered with ivermectin (Ivomec®) at 0.2 mg/kg (s.c.). Each point represents a mean; bars indicate the standard deviation (n=5-6). (*) $p \leq 0.05$

Figure 1

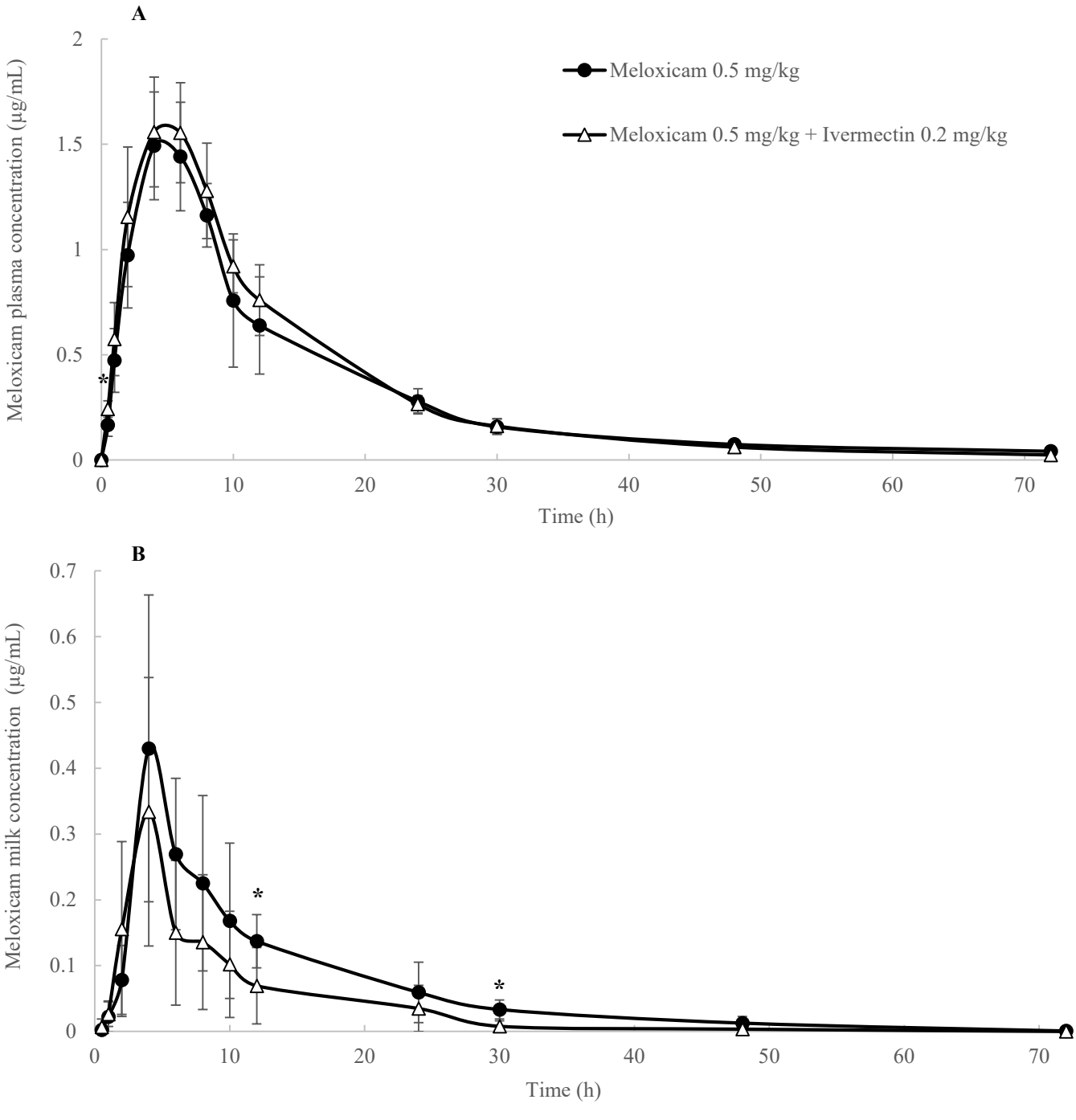

Table 1. Percentage of transport of meloxicam (30 µM) towards apical (BL-AP transport) or basal (AP-BL transport) compartments in MDCKII parental cells and the ovine-ABCG2 transduced cells in the absence or presence of ivermectin at 10 µM (n= 3-7).

		Time (h)	BL-AP (%transport)	AP-BL (%transport)	Ratio BL-AP/AP-BL
Meloxicam	MDCKII	2	30.71 ± 2.89	27.71 ± 2.43	
		4	38.59 ± 2.39	36.62 ± 2.62	1.06 ± 0.08
	MDCKII ovine ABCG2	2	43.31 ± 4.96	2.43 ± 1.40	
		4	62.87 ± 4.72	2.77 ± 0.75	24.85 ± 4.62[a]
Meloxicam + Ivermectin (10µM)	MDCKII	2	25.45 ± 1.09	19.30 ± 0.95	
		4	38.87 ± 1.85	27.09 ± 1.88	1.16 ± 0.04
	MDCKII ovine ABCG2	2	44.66 ± 2.30	5.66 ± 1.07	
		4	63.52 ± 3.38	10.36 ± 1.85	6.31 ± 1.37 [a,b]

Results are means ± SDs.
[a] $p \leq 0.05$, significant differences from parental MDCKII cells
[b] $p \leq 0.05$, significant differences from MDCKII ovine ABCG2 cells without ivermectin

Table 2. Mean (±SD) pharmacokinetic parameters of meloxicam in plasma and milk after subcutaneous administration at a dosage of 0.5 mg/kg in sheep co-administered with ivermectin (0.2 mg/kg s.c.) (n=5-6).

		Meloxicam 0.5 mg/kg	Meloxicam 0.5 mg/kg + Ivermectin 0.2 mg/kg
Plasma	$AUC_{(0-\infty)}$ (µg·h/mL)	24.3 ± 4.02	24.0 ± 2.87
	C_{max} (µg/mL)	1.53 ± 0.29	1.68 ± 0.15
	T_{max} (h)	4.33 ± 0.82	4.00 ± 0.00
	$T_{1/2}$ (h)	8.93 ± 1.38	8.90 ± 0.42
	MRT (h)	16.85 ± 0.85	14.60 ± 2.09
Milk	$AUC_{(0-\infty)}$ (µg·h/mL)	4.48 ± 0.89	2.72 ± 1.58*
	C_{max} (µg/mL)	0.48 ± 0.23	0.30 ± 0.21
	T_{max} (h)	4.33 ± 0.82	3.60 ± 1.67
	$T_{1/2}$ (h)	7.02 ± 4.34	5.03 ± 2.46
	MRT (h)	13.80 ± 4.05	9.24 ± 2.94
Milk/plasma	AUC	0.19 ± 0.03	0.11 ± 0.06*

* $p \leq 0.05$, significant differences from meloxicam 0.5 mg/k

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.