Role of eprinomectin as inhibitor of the ruminant ABCG2 transporter: Effects on plasma distribution of danofloxacin and meloxicam in sheep

Alba M. Garcia-Lino[a], Dafne Garcia-Mateos[a], Indira Alvarez-Fernandez[a], Esther Blanco-Paniagua[a], Juan M. Medina[a], Gracia Merino[a] and Ana I. Alvarez[a]

[a]Departmento de Ciencias Biomédicas, Fisiología, Facultad de Veterinaria. Instituto de Desarrollo Ganadero y Sanidad Animal (INDEGSAL), Universidad de León, Campus de Vegazana, León, Spain.

Corresponding author:

Dra. Ana I. Alvarez

Ciencias Biomédicas, Fisiología, Facultad de Veterinaria. Universidad de León

24071, Campus de Vegazana, León, Spain.

Phone: +34-987291265**; E-mail:** aialvf@unileon.es

Abstract

Therapeutic outcome results of the coadministration of several drugs in veterinary medicine is affected by, among others, the relationship between drugs and ATP-binding cassette (ABC) transporters, such as ABCG2. ABCG2 is an efflux protein involved in the bioavailability and milk secretion of drugs. The aim of this work was to determine the role of eprinomectin, a macrocyclic lactone (ML) member of avermectin class, as inhibitor of ABCG2. The experiments were carried out through in vitro inhibition assays based on mitoxantrone accumulation and transport assays in ovine ABCG2 transduced cells using the antimicrobial drug danofloxacin and the anti-inflammatory drug meloxicam, both widely used in veterinary medicine and well known ABCG2 substrates.

The inhibition results obtained showed that eprinomectin was an efficient in vitro ABCG2 inhibitor, tested in mitoxantrone accumulation assays. In addition, this ML decreased ovine ABCG2-mediated transport of danofloxacin and meloxicam. To evaluate the role of eprinomectin in systemic exposure of drugs, pharmacokinetic assays based on subcutaneous coadministration of eprinomectin with danofloxacin (1.25 mg/kg) or meloxicam (0.5 mg/kg) in sheep were performed obtaining a significant increase of systemic exposure of these drugs. Especially relevant was the increase of the systemic concentration of meloxicam, since coadministration with eprinomectin increased significantly the plasma concentration of meloxicam, obtaining an increase of AUC (0-72 h) value of more than 40%.

Keywords

ABCG2; danofloxacin; eprinomectin; macrocyclic lactone; meloxicam; sheep.

1. Introduction

Anthelmintic macrocyclic lactones (MLs) are antiparasitic drugs with notable broad-spectrum activity widely used for treatment of both internal and external parasites in animals and humans. Due to their lipophilicity, they are distributed throughout the body in the blood and lymph circulations, which produces long persistence in the host organism and consequently a long period of protection against parasite infection. Of special relevance is the semisynthetic ML eprinomectin, which is a drug derived from the natural product avermectin B1(abamectin), whose efficacy in goats and sheep is very high and, additionally, it is not transported into milk (Rostang et al., 2020).

Among other factors, the interactions between MLs and ATP-binding cassette (ABC) transporters control the systemic exposition of these drugs (Lespine et al., 2012; Virkel et al., 2018). MLs, including eprinomectin, strongly interact with P-glycoprotein (P-gp), the multidrug resistance 1 (MDR1) transporter (Lespine et al., 2012) which has been clearly identified as the main factor that controls the body concentration of MLs, such as ivermectin (Lespine et al., 2012; Merola and Eubig, 2018). Furthermore, milbemycins (such as moxidectin) and avermectins (such as ivermectin and doramectin) interact with the ABC transporter ABCG2 (Mealey, 2012; Real et al., 2011) which affects in vivo absorption, distribution and elimination of MLs. The coadministration of different drugs may promote alteration in disposition mediated by ABC transporters (Ballent et al., 2012; Mahnke et al., 2016; Virkel et al., 2018). ABCG2 expression in mammary gland is induced during lactation, and so plays an important role in the active secretion of many drugs into the milk of ruminants (García-Lino et al., 2019). The combination of drugs that interact with the ABCG2 transporter, including MLs, may affect their systemic exposure and their secretion into milk (Ballent et al., 2012).

The aim of the present study, therefore, was to characterize eprinomectin as an inhibitor of the ABCG2 transporter using two different ABCG2 substrates, the antimicrobial drug, danofloxacin (Real et al., 2011) and an anti-inflammatory drug, meloxicam (Garcia-Lino et al., 2020). The assays were performed in vitro using ABCG2 transduced ovine cells in a transepithelial transport and mitoxantrone accumulation assay, and in vivo using lactating Assaf sheep in a pharmacokinetics assay.

2. Materials and methods

2.1. *Reagents and drugs*

Mitoxantrone (MXR), eprinomectin, danofloxacin, meloxicam and difloxacin were purchased from Sigma-aldrich (St. Louis, MO). Isoflurane Isovet® was purchased from Braun (Barcelona, Spain). For the pharmacokinetic studies, eprinomectin solution (Eprecis®) was purchased from Ceva Salud Animal (Barcelona, Spain), danofloxacin solution (Advocin® 2.5 %) was purchased from Zoetis (Madrid, Spain) and meloxicam solution (Metacam® 20 mg/ml) was purchased from Boehringer Ingelheim.

2.2. Cell cultures

Madin-Darby Canine Kidney (MDCKII) cells were kindly provided by A. H. Schinkel (The Netherlands Cancer Institute, Amsterdam, The Netherlands). Stably-transduced MDCKII cells with ovine variant of ABCG2 were generated and characterized by our research group in previous studies (González-Lobato et al., 2014). Culture conditions were as previously described (González-Lobato et al., 2014).

2.3. Accumulation assay

In vitro accumulation assays were carried out as previously described (Pavek et al., 2005) using MXR as a fluorescent substrate.

Relative cellular accumulation of MXR of at least 5000 cells was determined by flow cytometry using a CyAn cytometer (Beckam Coulter, Fullerton, CA). Flow cytometry data were processed and analyzed using SUMMIT version 4.3 software (Innovation Drive, For Collins, CO). ABCG2 inhibition increases accumulation of MXR and thus increases MF. Inhibitory potencies of eprinomectin were calculated as previously described (Pavek et al., 2005) in MDKCII and oABCG2 cells according to the following equation: inhibitory potency = (MF with eprinomectin – MF without eprinomectin) / (MF with Ko143 – MF without eprinomectin).

2.4. Transport assays

Transepithelial transport assays using Transwell plates were carried out as described elsewhere (Perez et al., 2013) with minor modifications. The effect of eprinomectin as inhibitor in transport assay was tested using two ABCG2 substrates at concentrations used in previous in vitro studies, danofloxacin (10 μM) (Real et al., 2011) and meloxicam (30 μM) (Garcia-Lino et al., 2020). Parental MDCKII and ABCG2 ovine transduced subclones cells were grown for 3 days after seeding 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). Two hours before the start of the experiment, medium at both the apical and basolateral sides of the monolayer was replaced with 2 ml of OptiMEM medium, and either with or without the eprinomectin as inhibitor. The experiment was started (t= 0) by replacing the medium in either the apical or basolateral compartment with fresh OptiMEM medium, either with or without eprinomectin and containing meloxicam or danofloxacin. Cells were incubated at 37 ºC in 5% CO_2 and 100 μL aliquots of culture media were taken at 2 h and 4 h in the opposite compartment and this volume was replaced with fresh medium. The presence of danofloxacin or

meloxicam in the opposite compartment was presented as the fraction of total substrate added at the beginning of the experiment. Active transport across MDCKII monolayers was expressed by the relative transport ratio (R), defined as the percentage apically directed transport percentage divided by the percentage basolaterally directed translocation percentage, after 4 h.

2.5. Animals

Animals were housed on the Experimental Farm of the University of León, Spain, and handled according to institutional guidelines complying with European legislation. (2010/63/EU). Experimental procedures were approved by the Animal Care and Use Committee of the University of León and the Junta de Castilla y León ULE_011_2016 and ULE_008_2016.

2.6. Pharmacokinetic studies

Twenty-four lactating Assaf sheep (3–4 months in lactation) and weighing 70 to 85 kg were used. 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 SC injection at a therapeutic dose of (1.25 mg/kg) Advocin® (2.5%); (2) the second group (n=6) was injected SC with 1.25 mg/kg of Advocin® (2.5%) and co-administrated with a single SC dose of eprinomectin (Eprecis®) at 0.5 mg/kg based on previous studies (Rostang et al., 2020); (3) the third group (n=6) received a single SC injection at therapeutic dose (0.5 mg/kg meloxicam) of Metacam® (20 mg/mL); (4) and the fourth group (n=6) was injected SC with 0.5 mg/kg of Metacam® 20 mg/kg and co-administrated with a single SC dose of eprinomectin (Eprecis®) at 1 mg/kg based on previous studies (Lifschitz et al., 2008; Rehbein et al., 2014).

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, 1, 2, 3.75, 6.25, 8, 9.5, 12, 24, 32 and 48 h after danofloxacin administration and 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. Plasma and milk samples were stored at -20 ºC until HPLC analysis.

2.7. High Performance Liquid Chromatography

The chromatographic system used in samples analysis consisted of a Waters 2695 separation module and a Waters 2998 UV photodiode array detector.

Conditions for HPLC analysis of danofloxacin were modified in accordance with Perez et al. (2011). Standard samples in the appropriate drug-free matrix were prepared yielding a concentration range from 0.019 to 5 µg/ml, with coefficients of correlation > 0.99. The Limits of Quantification (LOQs) were 0.039 µg/ml for transport samples, 0.006 for plasma samples and 0.1 µg/ml for milk samples. The Limits of Detection (LODs) were 0.012 µg/ml for transport samples, 0.002 µg/ml for plasma samples and 0.042 µg/ml for milk samples. The extraction recovery levels for concentration in the standard curve were 82% for plasma and 85% for milk samples.

Conditions for HPLC analysis of meloxicam have been described previously (Garcia-Lino et al., 2020). Standard samples in the appropriate drug-free matrix were prepared yielding a concentration range from 0.019 to 15 µg/ml, with coefficients of correlation > 0.99. The LOQ was 0.01 µg/ml and the LOD was 0.005 µg/ml for transport samples; the LOQ was 0.02 µg/ml and the limit of LOD was 0.008 µg/ml for plasma samples; LOQ 0.02 µg/ml and LOD 0.007 µg/ml for milk samples. The extraction recovery levels for concentration in the standard curve were 88% for plasma and 90% for milk samples.

2.8. Pharmacokinetic calculations

The peak concentration (C_{max}) and time-peak concentration (T_{max}) were read from the plotted concentration-time curve for each animal. The area under concentration-time curves (AUC) from time zero to time of last sampling and to infinity were calculated using the trapezoidal method. Mean residence time (MRT) was calculated by the linear trapezoidal rule without extrapolation to infinity, using the formula: MRT = AUMC/AUC, where AUMC was the area under the momentum curve. These calculations were made using the PK Solutions computer program (Farrier, 1997) and determined by non-compartmental analyses.

2.9. Statistical analysis

Comparisons between groups were performed by 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). The results are shown as mean ± standard deviation (SD).

3. Results

3.1. Inhibitory potency of eprinomectin in mitoxantrone accumulation assays

To demonstrate the potential inhibitory effect of eprinomectin in ovine ABCG2, the ability of these compound to reverse the reduced MXR accumulation in cells transduced with ovine variant of ABCG2 was tested in flow cytometry experiments. As expected, MTX accumulation was significantly lower in the ovine variant of ABCG2 transduced cells compared to the parental cells because ABCG2 is actively transported this substrate outside the cells (Fig. 1). When cells were treated with Ko143, accumulation of MTX in the ovine variant of ABCG2 transduced cells increased by ABCG2

inhibition with Ko143 and thus increased the fluorescence to levels similar to those in the parental cells (Fig. 1). No significant differences between parental cells treated and non-treated with Ko143 inhibitor were observed.

The presence of eprinomectin inhibited ovine ABCG2, thus increasing the accumulation of MXR in ABCG2 transduced cell in a concentration dependent manner (Fig. 1). The highest inhibitory potency (63%) appeared at 10 μM for ovine ABCG2. Therefore, these results demonstrated that eprinomectin plays an important role as inhibitor of ovine ABCG2.

3.2. Inhibition of in vitro transport of danofloxacin and meloxicam by eprinomectin

To further characterise the inhibitory properties of the eprinomectin in ovine ABCG2, transepithelial transport assays were carried out with MDCKII cells transduced with the ovine variant of ABCG2 using a model substrate of ABCG2, danofloxacin (10 μM), and a new described ABCG2 substrate, meloxicam (30 μM) (Table 1 and 2, respectively). The translocation in MDCKII parental cells treated with danofloxacin at 10 μM in the apical and basolateral directions was similar, with a similar relative basal-apical:apical-basal transport ratio (Ratio BL-AP/ AP-BL close to 1, according with Real et al. (2011). In the ovine variant of ABCG2 transduced cells, translocation in the apical direction was drastically decreased and translocation in the basolateral direction was increased, resulting in a transport ratio BL-AP/ AP-BL of higher than 12 at 4 h (Table 1). A significant decrease in this relative transport ratio of danofloxacin, more than 80%, was observed in the ovine ABCG2-transduced cells when eprinomectin was added at a concentration of 5 μM (12.44 ± 6.89 vs 2.29 ± 0.25*). Ovine ABCG2 mediated transport was almost completely reverted at a concentration of 10 μM of eprinomectin with a relative transport ratio equal to that of the parental cells (1.08 ± 0.05 vs 0.86 ±

0.05). These differences were not observed in the parental MDCKII cells, thus indicating that the observed effect is ABCG2 specific.

Similar results were obtained in the transephitelial transport of meloxicam at 30 μM (Table 2). Relative efflux transport ratio at 4 h was significantly higher in the ovine ABCG2-transduced cells compared to the parental cells (24.85 ± 4.6* vs 1.06 ± 0.08). These results show that meloxicam is an in vitro substrate of the ovine ABCG2 variant. When eprinomectin was added, the apical to basal transport in cells transduced with ovine ABCG2 decreased compared to the cells without eprinomectin, presenting a reduction of 32% in the transport ratio of meloxicam in treatment with eprinomectin at 5 μM and of 78% at 10 μM. These differences were not observed in parental MDCKII cells.

These results clearly show that eprinomectin is a good in vitro inhibitor of ovine ABCG2.

3.3. Effect of eprinomectin on plasma pharmacokinetics and milk secretion of the antimicrobial danofloxacin and the anti-inflammatory drug meloxicam

To further demonstrate the in vivo ABCG2 inhibitory role of eprinomectin in clinically relevant drug-drug interactions, the effect of the co-administration of eprinomectin with danofloxacin and with meloxicam was studied in pharmacokinetic and milk secretion assays. A higher significant plasma concentration of both drugs, danofloxacin and meloxicam, was found in groups of animals coadministered with eprinomectin at several times: at 4 h after danofloxacin co-administration (Fig. 2) and at 6, 8, 10, 12, 24 and 30 h after meloxicam administration (Fig. 3). In addition, differences in plasma pharmacokinetic parameters were observed (Table 3 and Table 4). The value of AUC (0-48 h) increased significantly and was almost 1.3-fold higher in animals

coadministered with eprinomectin/danofloxacin compared with control animals. In sheep co-treated with meloxicam, plasma C_{max} and T_{max} were significantly higher for eprinomectin-treated animals compared to control animals. In addition, AUC (0-72 h) was almost 40% higher in animals co-administered with eprinomectin compared with the control group. No differences in milk concentration or pharmacokinetic parameters of danofloxacin and meloxicam were found (Tables 2 and 3; Figs. 2 and 3).

These results clearly show that the coadministration of eprinomectin influences the systemic distribution of danofloxacin and meloxicam without variation in milk drug concentration.

4. Discussion

Interaction with ABC transporters is recognized as a mechanism responsible for pharmacologically relevant in vivo drug-drug interactions. An unintentional result may be therapeutic failure or toxicity. However, a positive outcome is also possible and one drug may increase the systemic exposure of another one. Therefore, drug-drug interaction may occur after drug coadministration (Virkel et al., 2018). Interaction between commonly used drugs in veterinary medicine, such as fluoroquinolones antibacterial drugs, tyrosine kinase inhibitors and some anthelmintic benzimidazoles, with ABC transporters, including ABCG2, has been studied in depth (Barrera et al., 2013; Mealey, 2012; Virkel et al., 2018). In this study, eprinomectin is described for the first time as an efficient in vitro and in vivo inhibitor of ABCG2 transporter; drug-drug interaction mediated by ABCG2 is reported with the coadministration of this macrocyclic lactone and the ABCG2 substrates danofloxacin and meloxicam.

In the MXT accumulation assays, inhibitory potency higher than 50% (IC_{50}) was reported at 10μM in ovine ABCG2, which confirms eprinomectin as a good inhibitor

for ovine ABCG2 (Weiss et al., 2007). Other macrocyclic lactones have been described as in vitro ABCG2 inhibitors (Lespine et al., 2012). This role as inhibitor of eprinomectin was corroborated using transport assays testing interaction with danofloxacin, a model substrate of ABCG2, and meloxicam a novel substrate of ABCG2 (Garcia-Lino et al., 2020). In both cases, a reduction in the relative transport ratio was observed in ovine ABCG2-transduced cells versus cells in the presence of eprinomectin (Table 1 and 2). Eprinomectin has been previously described as a strong inhibitor of P-gp transporter (Lespine et al., 2012). Nevertheless, this is the first time that eprinomectin has been described as an in vitro ABCG2 inhibitor. Inhibition of in vitro transport of danofloxacin by macrocyclic lactones has been described previously with studies using ivermectin at 50 μM in human ABCG2- and murine Abcg2-transduced cells (Real et al., 2011). However, our results show that eprinomectin completely inhibits transport of danofloxacin mediated by ABCG2 in ovine ABCG2-trasduced cells at a 5-fold lower concentration (10 μM).

According to our positive results obtained in the in vitro assays, the extent of in vivo ABCG2-mediated drug-drug interaction involving eprinomectin and veterinary ABCG2 substrates has been determined in sheep (Figs. 2 and 3). Interaction between ABCG2 and eprinomectin was confirmed in an in vivo setting when eprinomectin was coadministered to sheep together with the antimicrobial danofloxacin (Fig. 2) and with the anti-inflammatory drug meloxicam. The pharmacokinetics of danofloxacin and meloxicam reported in this study were similar to those reported previously (Real et al., 2011; Woodland et al., 2019). Our results show that plasma availability, and consequently the therapeutic potential, of danofloxacin and meloxicam increases with the co-administration with eprinomectin (Figs. 2 and 3). An increase in plasma concentration of danofloxacin was previously observed with a combined administration

with ivermectin in sheep, causing an increase in plasma AUC values and half-life of this drug (Ballent et al., 2012). However, a decreased concentration of danofloxacin in milk was observed with the coadministration of ivermectin, a P-gp and ABCG2 inhibitor (Real et al., 2011). Our results after co-administration of both drugs with eprinomectin show no significant differences in concentration in milk compared with control animals. The low binding with lipoproteins that eprinomectin has compared to other MLs, such as ivermectin, may be among the causes of its low presence in milk (Lespine et al., 2012)and therefore in the mammary gland, probably reducing its ability to interact locally with ABCG2. It should be noted that the increase in plasma concentration of meloxicam produced by the co-administration of eprinomectin could have important therapeutic applications due to the therapeutic potential of meloxicam in small ruminants (Colditz et al., 2019).

In conclusion, the role of eprinomectin as an inhibitor of ovine ABCG2, both in vitro and in vivo, has been demonstrated. Co-administration of eprinomectin in sheep results in an increase in plasma concentration of the antimicrobial drug danofloxacin and the anti-inflammatory drug meloxicam, producing a synergic effect of these drugs. These new findings establish that eprinomectin, an effective antiparasitic, with no withdrawal period in milk, affects systemic exposure of other drugs by inhibition of ABCG2 transporter.

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Authorship contributions

Participated in the research design: Merino, Alvarez, Garcia-Lino.

Conducted experiments: Garcia-Lino, Blanco-Paniagua, Astorga-Simon, Garcia-Mateos, Alvarez-Fernandez, Medina.

Performed data analysis: Merino, Alvarez, Garcia-Lino.

Wrote or contributed to the writing of the manuscript: Alvarez, Merino, Garcia-Lino.

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Table 1. Percentage of transport of danofloxacin (10 µM) towards apical (BL-AP transport) or basal (AP-BL transport) compartments in MDCK parental cells and their ovine ABCG2-transduced cells in the absence or presence of eprinomectin at 5 µM or 10 µM (n= 4–7).

		Time(h)	BL-AP (% transport)	AP-BL (% transport)	Ratio BL-AP/ AP-BL
Danofloxacin	MDCKII	2	14.72 ± 1.59	15.02 ± 3.42	
		4	25.76 ± 1.86	26.44 ± 4.09	0.98± 0.09
	MDCKII ovine ABCG2	2	39.54 ± 4.42	2.76 ± 2.86	
		4	58.65 ± 3.39	5.58 ± 2.13	12.44 ± 6.89[a]
Danofloxacin + Eprinomectin (5µM)	MDCKII	2	12.20 ± 1.02	9.88 ± 1.96	
		4	20.05 ± 1.18	22.09 ± 0.86	0.91 ± 0.03
	MDCKII ovine ABCG2	2	21.74 ± 0.89	5.97 ± 2.69	
		4	32.87 ± 3.74	14.38 ± 0.39	2.29 ± 0.25[b]
Danofloxacin + Eprinomectin (10µM)	MDCKII	2	11.06 ± 1.28	13.82 ± 3.50	
		4	21.93 ± 4.60	25.74 ± 5.85	0.86 ± 0.05
	MDCKII ovine ABCG2	2	12.06 ± 1.68	9.65 ± 0.49	
		4	24.36 ± 4.46	22.57 ± 4.87	1.08 ± 0.05[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 eprinomectin.

Table 2.

Percentage of transport of meloxicam (30 μM) towards apical (BL-AP transport) or basal (AP-BL transport) compartments in MDCK parental cells and their ovine-ABCG2 transduced cells in the absence or presence of eprinomectin at 5 μM or 10 μM (n= 4–7).

		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 + Eprinomectin (5μM)	MDCKII	2	29.34 ± 0.49	21.83 ± 2.58	
		4	38.32 ± 2.02	35.36 ± 1.49	1.08 ± 0.01
	MDCKII ovine ABCG2	2	50.70 ± 1.44	3.14 ± 0.36	
		4	66.01 ± 4.74	4.27 ± 0.57	15.69 ± 3.21[b]
Meloxicam + Eprinomectin (10μM)	MDCKII	2	25.11 ± 3.96	17.72 ± 0.96	
		4	36.70 ± 1.55	33.00 ± 1.36	1.11 ± 0.04
	MDCKII ovine ABCG2	2	43.61 ± 0.98	6.94 ± 2.99	
		4	62.40 ± 3.93	13.81 ± 6.61	5.33 ± 2.35[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 eprinomectin.

Table 3.

Mean (±SD) pharmacokinetic parameters in plasma of sheep after subcutaneous administration of danofloxacin at a dosage of 1.25 mg/kg in sheep coadministered with eprinomectin (0.5 mg/kg s.c.)

		Danofloxacin	Danofloxacin + Eprinomectin
Plasma	AUC (0-48 h)	1.40 ± 0.04	1.80 ± 0.23*
	Cmax (µg/mL)	0.16 ± 0.05	0.2 ± 0.06
	Tmax (h)	2.67 ± 0.93	2.27 ± 1.26
	MRT (h)	9.66 ± 3.22	9.15 ± 2.39
Milk	AUC (0-48 h)	16.2 ± 2.96	15.41 ± 1.79
	Cmax (µg/mL)	2.00 ± 0.81	1.88 ± 0.37
	Tmax (h)	2.66 ± 2.08	4.5 ± 1.71
	MRT (h)	6.38 ± 0.32	6.7 ± 0.49
Milk/plasma	AUC	11.9 ± 4.49	10.19 ± 2.51

* $p \leq 0.05$, significant differences from control group

Table 4.

Mean (±SD) pharmacokinetic parameters in plasma of sheep after subcutaneous administration of meloxicam at a dosage of 0.5 mg/kg in sheep coadministered with eprinomectin (1.0 mg/kg s.c.)

		Meloxicam	Meloxicam + Eprinomectin
Plasma	AUC (0-72 h)	23.7 ± 3.94	33.1 ± 6.77*
	Cmax (µg/mL)	1.53 ± 0.29	1.83 ± 0.24*
	Tmax (h)	4.33 ± 0.82	6.00 ± 0.00*
	MRT (h)	16.9 ± 0.85	17.6 ± 3.89
Milk	AUC (0-72 h)	4.33 ± 0.92	5.13 ± 1.87
	Cmax (µg/mL)	0.48 ± 0.23	0.37 ± 0.08
	Tmax (h)	4.33 ± 0.82	4.50 ± 1.97
	MRT (h)	13.8 ± 4.05	18.08 ± 7.68
Milk/plasma	AUC	0.19 ± 0.03	0.17 ± 0.02

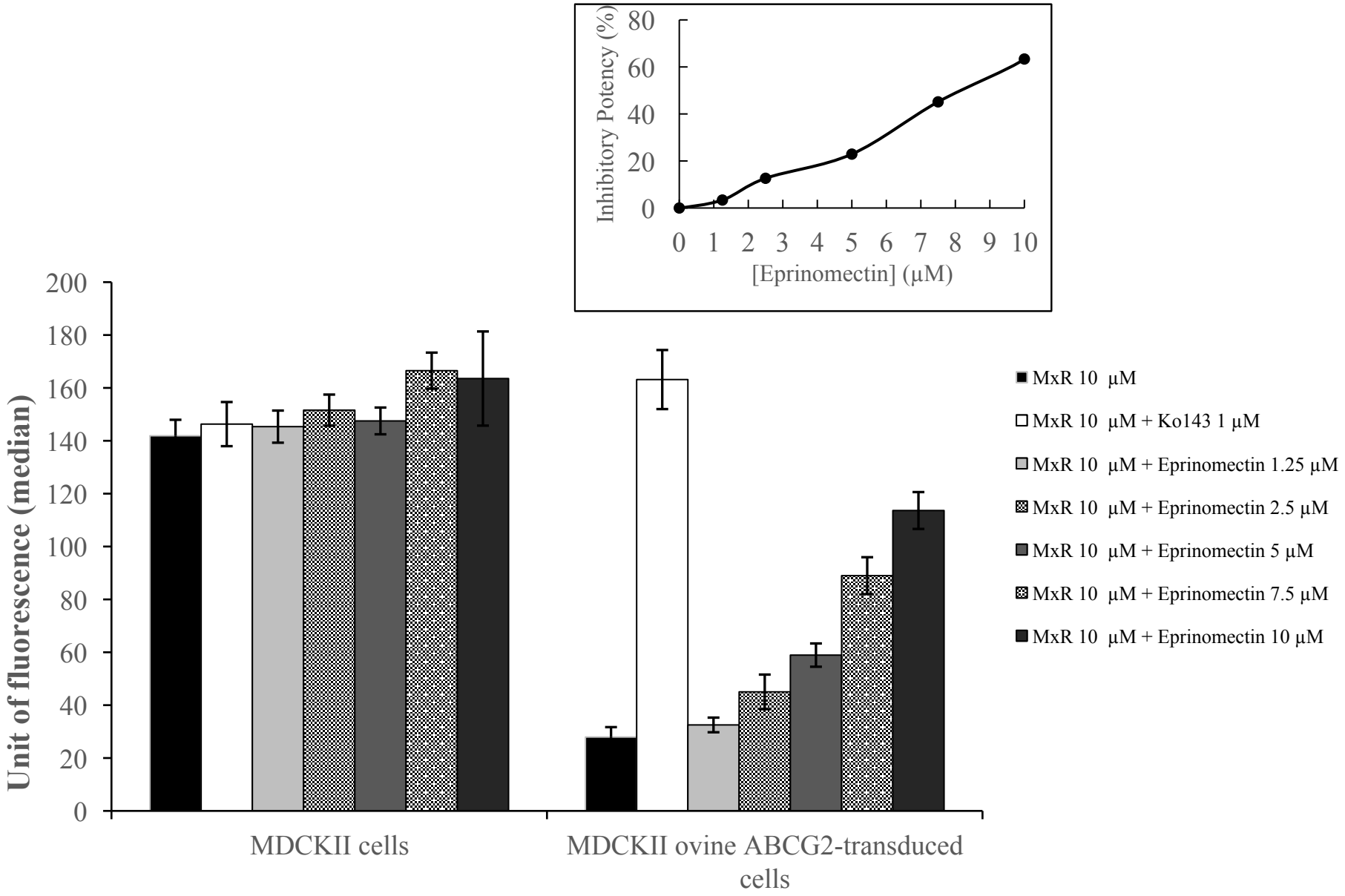
* $p \leq 0.05$, significant differences from control group

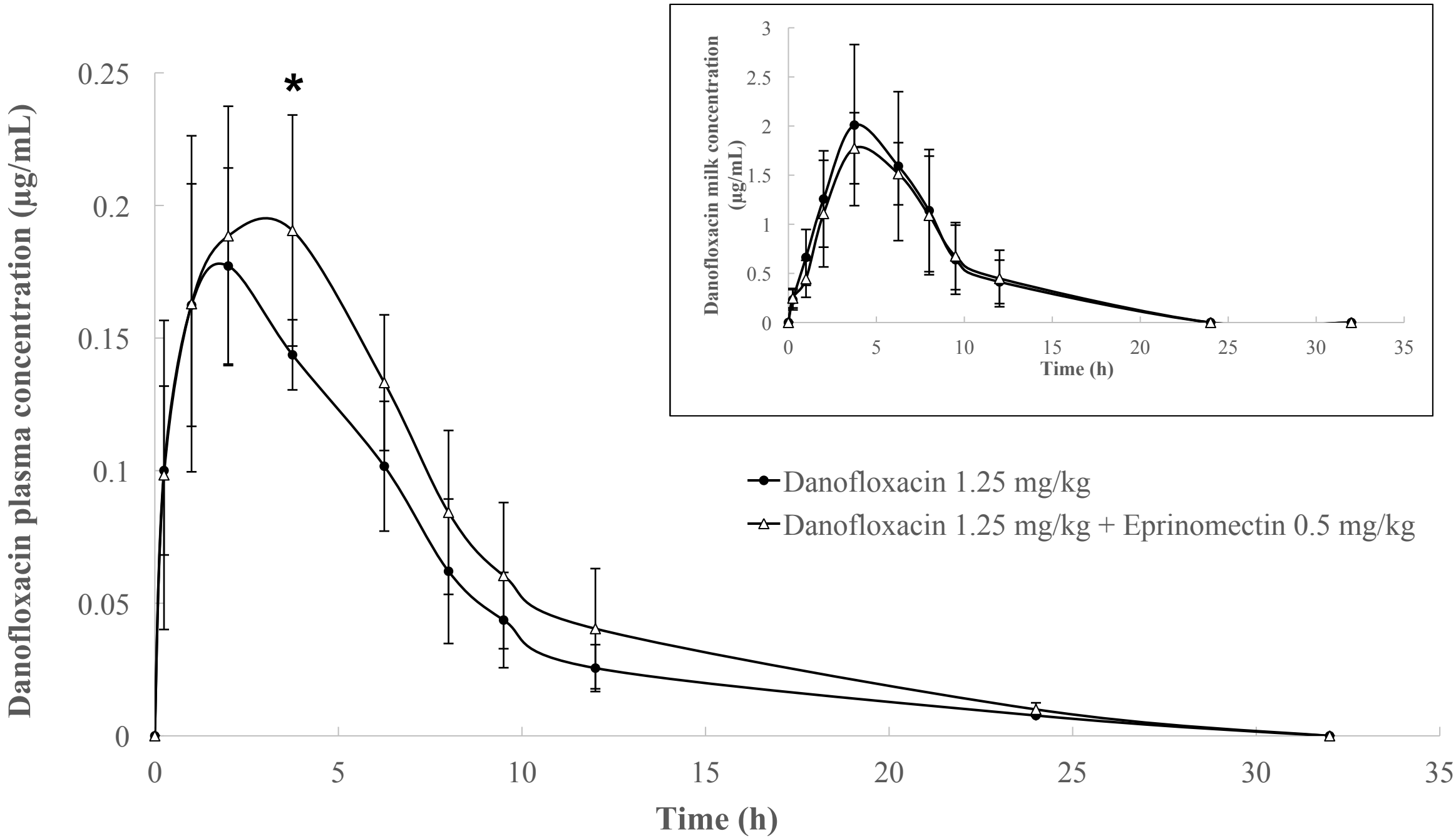
Figures legends

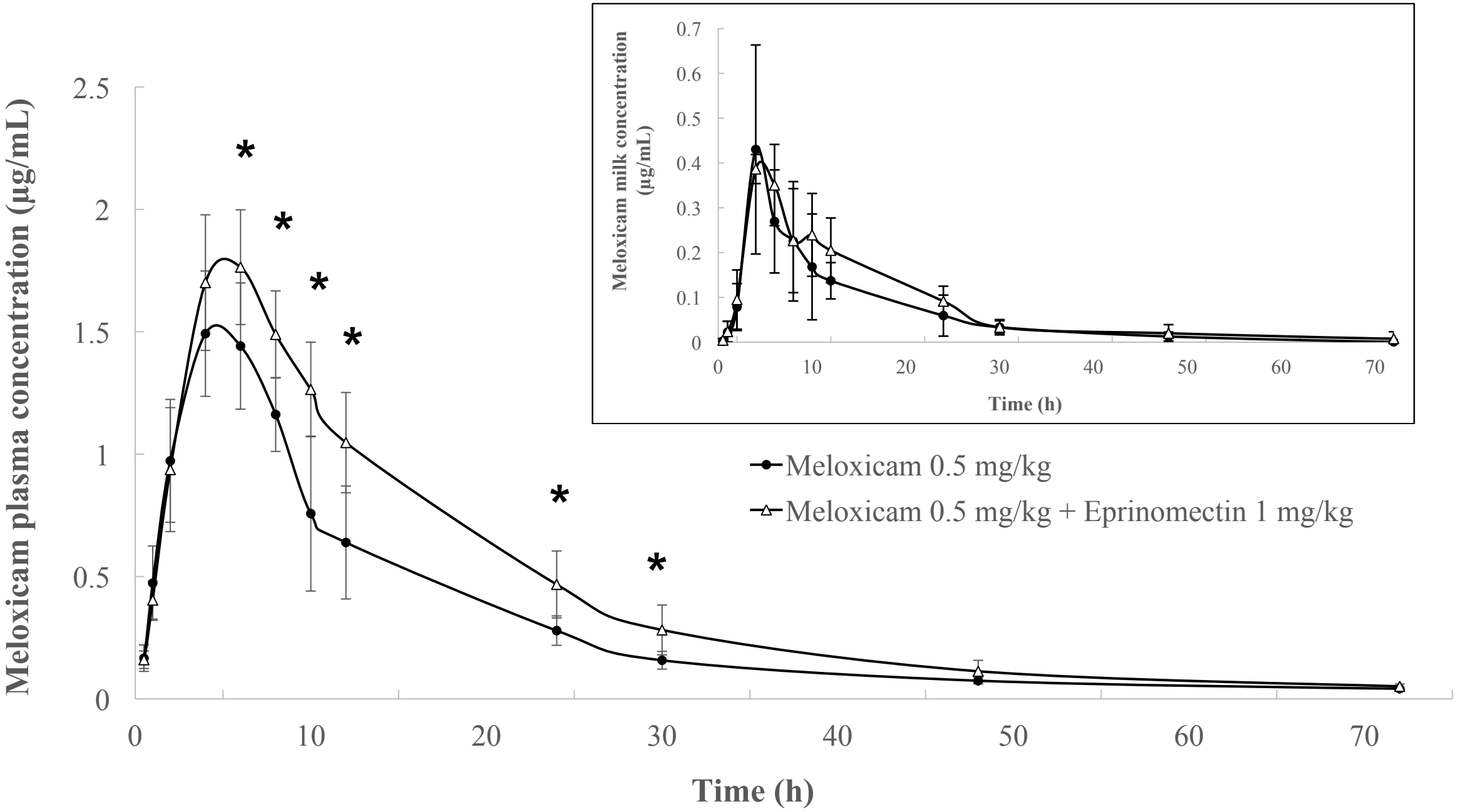
Fig 1. Effect of eprinomectin on accumulation of mitoxantrone (10 μM) at 1.25, 2.5, 5, 7.5 or 10 μM in parent MDCKII cells and in their ovine ABCG2 transduced cells. Results (units of fluorescence, median); error bars indicate SD. (n=3-6). Inhibitory potency (%) of eprinomectin in ovine ABCG2-transduced cells is also represented.

Fig 2. Concentrations in plasma and milk (embedded) vs. time curves for danofloxacin obtained from lactating Assaf sheep treated with a single dose of Advocin® at 1.25 mg/kg (sc) and coadministered with Eprecis® at 0.5 mg/kg (sc). Each point represents a mean; bars indicate standard deviation (n=5-6). (*) $p \leq 0.05$.

Fig 3. Concentrations in plasma and milk (embedded) vs. time curves for meloxicam obtained from lactating Assaf sheep treated with a single dose of Metacam® at 0.5 mg/kg (sc) and co-administered with Eprecis® at 1 mg/kg (sc). Each point represents a mean; bars indicate standard deviation (n=5-6). (*) $p \leq 0.05$.


Inhibitory Potency (%)
80
60
40
20
0
0 1 2 3 4 5 6 7 8 9 10
[Eprinomectin] (μM)
Unit of fluorescence (median)
200
180
160
140
120
100
80
60
40
20
0
MDCKII cells
MDCKII ovine ABCG2-transduced cells
MxR 10 μM
MxR 10 μM + Ko143 1 μM
MxR 10 μM + Eprinomectin 1.25 μM
MxR 10 μM + Eprinomectin 2.5 μM
MxR 10 μM + Eprinomectin 5 μM
MxR 10 μM + Eprinomectin 7.5 μM
MxR 10 μM + Eprinomectin 10 μM


Danofloxacin plasma concentration (µg/mL)
0.25
0.2
0.15
0.1
0.05
0
*
0
5
10
15
20
25
30
35
Time (h)
Danofloxacin 1.25 mg/kg
Danofloxacin 1.25 mg/kg + Eprinomectin 0.5 mg/kg
Danofloxacin milk concentration (µg/mL)
3
2.5
2
1.5
1
0.5
0
0
5
10
15
20
25
30
35
Time (h)


Meloxicam plasma concentration (µg/mL)
2.5
2
1.5
1
0.5
0
0
10
20
30
40
50
60
70
Time (h)
*
*
*
*
*
*
Meloxicam 0.5 mg/kg
Meloxicam 0.5 mg/kg + Eprinomectin 1 mg/kg
Meloxicam milk concentration (µg/mL)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
10
20
30
40
50
60
70
Time (h)