Received: 7 June 2022 | Revised: 7 July 2022 | Accepted: 17 July 2022

DOI: 10.1111/jvp.13089

JOURNAL OF Veterinary Pharmacology and Therapeutics WILEY

ORIGINAL ARTICLE

Robenacoxib pharmacokinetics in sheep following oral, subcutaneous, and intravenous administration

Charbel Fadel[1] | Beata Łebkowska-Wieruszewska[2] | Irene Sartini[3] | Andrzej Lisowski[4] | Amnart Poapolathep[5] | Mario Giorgi[1,3]

[1]Department of Veterinary Medicine, University of Sassari, Sassari, Italy

[2]Department of Pharmacology, Toxicology and Environmental Protection, University of Life Sciences, Lublin, Poland

[3]Department of Veterinary Sciences, University of Pisa, Pisa, Italy

[4]Institute of Animal Breeding and Biodiversity Conservation, University of Life Sciences, Lublin, Poland

[5]Faculty of Veterinary Medicine, Department of Pharmacology, Kasetsart University, Bangkok, Thailand

Correspondence
Charbel Fadel, Department of Veterinary Medicine, University of Sassari, Sassari, Italy.
Email: c.fadel@studenti.uniss.it

Funding information
Università di Pisa; University of Pisa

Abstract

The aim of this study was to evaluate the pharmacokinetics (PK) of robenacoxib (RX), a COX-2 selective non-steroidal anti-inflammatory drug, in sheep after single subcutaneous (SC), oral (PO), and intravenous (IV) administration. Five healthy female sheep underwent a three-phase parallel study design with a washout period of 4 weeks, in which sheep received a 4 mg/kg SC dose in phase 1, a 4 mg/kg PO administration in phase 2, and a 2 mg/kg IV administration in phase 3. Plasma RX concentrations were measured over a 48 h period for each treatment using HPLC coupled to a UV multiple wavelength detector, and the PK parameters were estimated using a non-compartmental method. Following IV administration, terminal elimination half-life, volume of distribution at steady state, and total clearance were 2.64 h, 0.077 L/kg, and 0.056 L/h kg, respectively. The mean peak plasma concentrations following SC and PO administrations were 7.04 and 3.01 μg/mL, respectively. The mean bioavailability following SC and PO administrations were 45.98% and 16.58%, respectively. The SC route may be proposed for use in sheep. However, the multi-dose and pharmacodynamic studies are necessary to establish more accurately its safety and efficacy in sheep.

KEYWORDS
coxib, cyclooxygenase, nonsteroid anti-inflammatory, pain management, pharmacokinetics, robenacoxib, sheep

1 | INTRODUCTION

Changing moral and ethical considerations have led to societal demands for better agricultural practices and enhanced wellbeing for food producing animals all around the world. In this perspective, proper pain management is a critical component of promoting farm animal welfare. Pain alters behavior, autonomic, and neuroendocrine function. It causes a depressed mood and is a common cause of animal welfare violations (Steagall et al., 2021). In farm animals, chronic pain, for example, was shown to reduce food consumption and average daily weight gain, raises heart rate and blood pressure, and lowers body temperature (Stewart et al., 2010).

Sheep are subjected to various husbandry operations such as castration, vasectomy, and tail docking, and are prone to developing painful pathologies such as lameness, mastitis, vaginal prolapse, and penis deviation. Moreover, sheep are also widely employed as an experimental animal model for particularly invasive surgeries (Coulter et al., 2009), for educational purposes, and biological research (Lizarraga & Chambers, 2012).

There are several reasons for the inadequate pain management in small ruminants. For instance, in the United States and Europe, there are no non-steroidal anti-inflammatory drugs (NSAIDs) approved for the use in managing pain in sheep or goats. (Lizarraga & Chambers, 2012; Smith et al., 2021). As a result, these medications are being utilized off-label. Furthermore, this off-label use is often limited by the paucity of knowledge regarding the pharmacokinetics, efficacy and residue depletion of the drug in these animal species. The difficulty in administering injectable drugs in some cases, alongside cost and time, are other reasons on the list too (Huxley & Whay, 2007; Lizarraga & Chambers, 2012). It should be noted that a few NSAIDs for sheep and goats are approved in some nations, such as Canada, New Zealand, Australia, India, Mexico, Peru, and Costa Rica (Anonymous, 2016; 2020; Turk et al., 2021).

Non-steroidal anti-inflammatory medications are widely used in veterinary and human medicine for their anti-inflammatory, analgesic, and anti-pyretic actions. In sheep, the analgesic efficacy of this class of drugs has been frequently reported, such as for sheep suffering from footrot or undergoing castration and tail-docking (Small et al., 2014; Welsh & Nolan, 1995).

Nevertheless, non-selective NSAIDs may involve adverse side effects. COX-1 is present in many tissues constitutively and has several protective physiological functions, including gastric cytoprotection, and regulation of both renal blood flow and platelet activity. Differently, COX-2 is mainly induced locally and for restricted periods and is primarily responsible for inflammation and pain (Fadel et al., 2021; Pairet & Engelhardt, 1996). Therefore, NSAIDs that inhibit COX-2 but spare COX-1 were designed to have improved safety margins (Flower, 2003). Consequently, a new class of drugs named COXIBs, selective COX-2 inhibitors, has appeared on the market. Among this class, robenacoxib (RX) is a highly selective COX-2 inhibitor and is registered as injectable and flavored tablet formulations for dogs and cats (EMA, 2008). In all the targeted species and at clinically recommended dosages, no significant COX-1 inhibition was observed (in vitro IC50 ratios COX-1:COX-2, 129:1 in dogs, 32:1 in cats) (Lees et al., 2022; Schmid, Seewald, et al., 2010). Animal species other than dogs and cats, such as sheep, could potentially benefit from this drug. However, the pharmacokinetic (PK) and pharmacodynamic (PD) differences among animal species, especially between ruminants and monogastric species, require studies to elucidate the behavior of the drug in the target species. To the best of our knowledge, there are no reported RX studies in sheep. Hence, the aim of this study was to determine the pharmacokinetics of RX following a single oral (PO, 4 mg/kg), subcutaneous (SC, 4 mg/kg), and intravenous (IV, 2 mg/kg) dose.

2 | MATERIALS AND METHODS

2.1 | Chemicals and reagents

The pure powders of RX and diclofenac as internal standard (IS) with a standard purity of 99.0%, alongside the sodium chloride (NaCl), were purchased from Sigma-Aldrich (Milan, Italy). High performance liquid chromatography (HPLC)-grade acetonitrile (ACN), methanol (MeOH), and formic acid were obtained from VWR chemicals (Oud-Heverlee, Belgium). Deionized water was produced using a Milli-Q Millipore Water System (Millipore, Darmstadt, Germany). The aqueous and organic components of the mobile phase were degassed under pressure and mixed in the HPLC system. The mobile phases were filtered through 0.2 μm cellulose acetate membrane filters (Sartorius Stedim Biotech, Goettingen, Germany) with a solvent filtration apparatus.

2.2 | Animals and experimental design

The study employed five healthy adult female sheep (Wrzosówka breed) with body weights ranging from 18 to 26 kg (10–14 months of age). This experiment was carried out at the University of Life Sciences in Lublin, Poland.

The sheep were monitored daily through observation of behavior and appetite. They were acclimatized in an animal shed for 7 days prior to the start of the trial. Ad libitum feed (alfalfa hay) and water were provided, and animals were able to graze freely during the day as ear tags with an identity code were applied to the left ear, for easier identification.

The animal experiment was approved by the University of Lublin's animal welfare ethics committee and conducted in compliance with European law (Directive 2010/63/EU).

2.3 | Drug

The commercial SC formulation containing 20 mg RX per ml (Onsior®, Elanco, Italy), and the oral tablets of 40 mg each (Onsior®, Elanco, Italy), were used in this study. Due to the fact that no previous data were published in ruminants, the selected doses were based on RX data present in cats and dogs, for which the Onsior® tablets are approved in the European Union for surgical applications at a dose of 2 mg/kg, with a range of 2–4 mg/kg (EMA, 2008).

2.4 | Drug dosing and sample collection

Animals underwent a three-phase parallel study design, with a washout period of 4 weeks to ensure an adequate clearance of the drug. The sheep were weighed each day before administration, and the doses were adjusted correspondingly. In phase I, a SC injection of 4 mg/kg RX was performed behind the right shoulder, above the ribs. In phase 2, the 4 mg/kg PO doses were prepared by carefully partitioning and weighing the grinded tablets of RX. The tablets were then dissolved in 20 ml of water and administered via an ororuminal tube, immediately after which the tube was flushed with 400 ml of water. In the third phase, sheep received a slow IV injection of RX at a dose of 2 mg/kg, in the right jugular vein.

Blood samples were collected using vacutainer lithium heparin tubes (BD, Vaud, Switzerland) from the left jugular vein at 0, 0.085 (for IV only), 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 6, 8, 10, 24, and 48 h. Blood was centrifuged for 10 min at 1500 *g* immediately after collection. Then, the plasma was harvested, transferred in crio-vials, and stored at −20°C. It was analyzed within 4 weeks of each phase of the study.

2.5 | Sample preparation

The procedure was determined using a modified published method (Jung et al., 2009). In brief: to 200 µl of plasma, 50 mg of NaCl was added to increase the ionic power of water. Plasma then was spiked with 50 µl of IS (50 µg/ml) solution in MeOH. Afterward, 800 µl of ACN was added. After thorough vortex mixing (30 s), the samples were shaken at 60 oscillations/min for 10 min and centrifuged at 4000*g* for 10 min. The upper layer was transferred into a clean tube and dried at 45°C under a gentle nitrogen stream. The residue was dissolved in 120 µl of ACN:water 60:40 (v/v), vortexed for 1 min, sonicated at 25°C for 10 min, and then finally centrifuged at 4000*g* for 2 min. An aliquot of 50 µl of the upper layer was injected onto the HPLC system for analysis.

2.6 | HPLC conditions

The HPLC system was a LC Jasco consisting of a ternary gradient system (PU 980), in line degasser (DG-2080-53), autosampler (AS2055), and an UV multiple wavelength detector (MD-1510). The chromatographic separation assay was performed with a Luna C18 analytical column (150 × 4.6 mm inner diameter, 3 µm particle size, Phenomenex) maintained at 30°C using a Peltier system (CO4062) (Jasco). The mobile phases were 0.1% v/v formic acid in water:ACN 95:5 (v/v) (phase A) and ACN (phase B). The column was eluted isocratically using 38% A and 62% B at a flow rate of 1 ml/min. The optimal wavelength for the quantification was set at 275 nm.

2.7 | Validation of the analytical method

RX (1 mg/ml) and IS (1 mg/ml) stock solutions were produced in MeOH, and were diluted to reach a concentration of 50 µg/ml, and then stored at −20°C. This last concentration was then diluted to the following concentrations: 10, 5, 2.5, 1, 0.5, 0.1, and 0.05 µg/ml, in order to prepare the calibration curve of RX in plasma. Spiked curves were constructed with these RX concentrations versus ratio of IS peak areas. Linearity of the calibration curves, in the range of 0.05–50 µg/ml for plasma, was assessed based on the residual plot, fit test, and back calculation. The intra-day and the inter-day precision were calculated after analysis of six plasma samples spiked with IS at high (10 µg/ml), middle (1 µg/ml), and low (0.05 µg/ml) concentration standards (quality control QC samples), with the same instrument, the same operator in the same day and in three different days, respectively. These precision values were expressed as the percentage coefficients of variation (CV, %). The recoveries of the drug were evaluated by comparison with the detector responses obtained for the extracted quality control samples and those for the pure standards dilutions. The recovery was expressed as mean ± standard deviation (SD). The limit of detection (LOD) was estimated as the plasma concentration that produced a signal to noise ratio of 3, and the lower limit of quantification (LLOQ) was determined as the lowest plasma concentration that produced a signal to noise ratio of 5. The analytes were stable for at least 16 weeks if stored at −20°C.

2.8 | Pharmacokinetic analysis

The data were pharmacokinetically analyzed using a non-compartmental approach (ThothPro™T 4.3; ThothPro LLC, Poland). The maximum plasma concentration (C_{max}) and time to reach it (T_{max}) were determined directly from the concentration vs. time curves. The elimination half-life (t1/2λz) was calculated using least squares regression analysis of the concentration-time curve. The area under the curve (AUC) was calculated by linear log trapezoidal (IV administration) and the linear-up log-down rule (PO and SC administration). Area under the first moment curve (AUMC) was calculated as $\int_0^\infty 0$ C(t)dt. From these values, the volume of distribution at steady state (Vss = dose × AUMC/AUC^2), mean residence time (MRT = AUMC/AUC), and clearance (Cl = dose/AUC) were calculated. The individual value of $AUC_{rest\%}$ was lower than 20% of $AUC_{(0-\infty)}$, and the square of coefficient of determination (R^2) of the terminal phase regression line was >0.85. Values below the LLOQ were not considered for the pharmacokinetic analysis.

The PO and SC bioavailability (F) were calculated using the following equation:

$$F\% = 100 \times \frac{\text{AUC(SC or PO)} \times \text{Dose (IV)}}{\text{AUC(IV)} \times \text{Dose (SC or PO)}}$$

The mean absorption time (MAT) was calculated using the following equation:

$$\text{MAT(PO or SC)} = \text{MRT(PO or SC)-MRT(IV)}.$$

The extraction ratio (E) for RX after IV administration was calculated for sheep as the clearance divided by cardiac output, where cardiac output (mL/kg/min) was calculated as body weight (kg) to the power of −0.19 multiplied by 180 (Toutain & Bousquet-Mélou, 2004a, 2004b).

$$E\% = \frac{\text{Body clearance}}{\text{Cardiac output}} = \frac{\text{Body clearance}}{180 \times \text{Body weight}^{-0.19}}$$

2.9 | Statistical analysis

Bonferroni's multiple comparison test (repeated measures ANOVA) was used to determine statistically significant differences of pharmacokinetic variables between the three treatment groups. As to compare

T_{max}, C_{max}, F%, and MAT between the SC and PO groups, paired t-test was used. The pharmacokinetic parameters are reported as geometric mean and ranges, except for T_{max} (categorical variable) which is expressed as the median value and range (Julious & Debarnot, 2000). The p value <0.05 was considered statistically significant. The analyses were conducted using GraphPad InStat (GraphPad Software 5.3v).

3 | RESULTS

3.1 | Validation of the method

The quantitative HPLC method was fully validated for sheep plasma in terms of linearity, intra-day and inter-day precision, selectivity, recovery, LOD, and LLOQ, according to the EMA guidelines (Anonymous, 2012). The selectivity of the method was checked for interference with blank plasma and spiked samples, where no peaks interfering with RX were observed. The analytical method demonstrated optimal linearity, with R^2 of 0.999 (y = 0.1223x + 0.003). The LOD and LLOQ were 0.01 and 0.05 μg/mL, respectively, and the mean extraction recovery was 95% ± 14%. The inter- and intra-day precision showed a CV% lower than 14.3% and 2.69%, respectively. The mean concentrations were <15% of the nominal values for the QCs and LLOQ samples.

3.2 | Animals

The sheep were judged to be clinically healthy after a physical examination as well as extensive chemical and hematological testing. A qualified veterinarian (B L-W) examined them and determined that they were healthy, that no recent pharmacological treatment had been administered, and that the sheep were parasite-free. They did not exhibit visible immediate or delayed (up to 7 days) local or systemic adverse effects as well.

3.3 | Pharmacokinetics

The mean (±SD) plasma concentrations of RX at the times of sample collection after IV, SC, and PO administration are plotted in Figure 1. The mean pharmacokinetic parameters, based on non-compartmental pharmacokinetic analysis, are presented in Table 1. RX was detected in plasma up to 24 h in all routes of administration (traces), however, it was quantifiable only until 10 h. The mean bioavailability was determined to be moderate following SC administration (45.98%), and low following PO administration (16.58%). Accordingly, the $AUC_{(0-\infty)}$, corrected for the dose, revealed statistically significant changes according to the administration route, with an order of IV > SC > PO. After IV administration, the mean calculated Cl was slow (0.056 L/kg h), and the Vss was low (0.077 L/kg). The E value had an average of 0.01. Moreover, the $MRT_{(0-\infty)}$ was not statistically different between SC and PO, however, statistically different for the IV route compared with both of SC and PO ($p < 0.05$).

4 | DISCUSSION

To the best of our knowledge, this is the first study which reports the pharmacokinetics of RX in sheep. An ideal anti-inflammatory and pain medication for pets and production animals would be safe, easy to administer, well-absorbed, and have a relatively long half-life and effect allowing for less frequent dosing (Stuart et al., 2019). The present study was conducted to determine the pharmacokinetics of RX when administered IV, SC, and PO.

The dose of RX differed between IV and the extravascular routes in the present study. To avoid toxicity issues and collateral effects, the IV dose was purposefully chosen lower than for the other routes of administration. Furthermore, although IV is not an approved route of administration of RX, IV pharmacokinetic study was performed to establish disposition kinetic variables, such as Vss, Cl, and F. Although dose-dependent pharmacokinetics cannot be excluded, RX was found to be independent of dose with linear plasma RX concentrations in dogs (Borer et al., 2017; King et al., 2011; Schmid, Spreng, et al., 2010). Additionally, despite administration of a higher PO dose, the peak plasma concentrations achieved were still less than those achieved after IV administration and the plasma concentrations on the terminal portions of the curves were similar for IV, PO, and SC administration. Given the observations in our study, and the linearity of RX concentrations observed in dogs, the use of

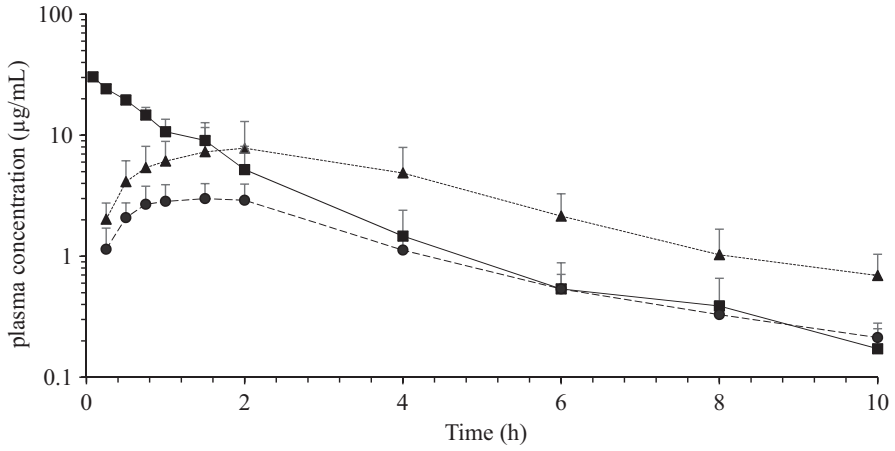

FIGURE 1 Semi logarithmic mean plasma concentration–time curves of robenacoxib following intravenous (IV, 2 mg/kg, —■—), subcutaneous (SC, 4 mg/kg, ---▲---), and oral (PO, 4 mg/kg, --●--) administrations in sheep ($n = 5$)

TABLE 1 Mean pharmacokinetic parameters and range in sheep ($n = 5$) after single IV (2 mg/kg), SC (4 mg/kg), and PO (4 mg/kg) doses of robenacoxib

	IV				SC			PO		
Parameter	**Unit**	**Geo mean**	**Min**	**Max**	**Geo mean**	**Min**	**Max**	**Geo mean**	**Min**	**Max**
$AUC_{(0-t)}$	mgh/L	36.02[b,c]	25.43	52.9	31.81[a,c]	18.55	64.03	11.03[a,b]	7.95	15.73
$AUC_{(0-\infty)}$ normalized	mgh/L	71.3[b,c]	50.06	103.1	33.63[a,c]	19.94	66.58	11.82[a,b]	8.61	16.43
λz	1/h	0.259	0.181	0.352	0.318	0.263	0.401	0.258	0.222	0.292
t1/2λz	h	2.64	1.84	3.82	2.18	1.73	2.63	2.69	2.37	3.12
Cl	L/kg/h	0.056	0.038	0.079	/	/	/	/	/	/
Vss	L/kg	0.077	0.065	0.088	/	/	/	/	/	/
$MRT_{(0-t)}$	h	1.4[b,c]	0.96	1.78	3.27	2.76	3.78	3.02	2.78	3.22
$MRT_{(0-\infty)}$	h	1.66[b,c]	1.14	1.97	3.79	2.99	4.55	3.75	3.24	4.03
C_{max}	µg/mL	/	/	/	7.04	4.28	16.49	3.01	2.21	4.48
T_{max}[d]	h	/	/	/	2	1	2	1.5	0.75	2
F	%	/	/	/	45.98[c]	31.36	71.72	16.58	13.71	19.46
MAT	h	/	/	/	1.87	1.8	2	1.62	1.82	1.44

Note: $AUC_{(0-t)}$, area under the curve from 0 h to last time collected samples; $AUC_{(0-\infty)}$, area under the curve from 0 h to infinity; λz, terminal phase rate constant; t1/2λz, terminal half-life; Cl, plasma clearance; Vss, volume of distribution; $MRT_{(0-t)}$, mean residence time from 0 h to last time collected samples; $MRT_{(0-\infty)}$, mean residence time from 0 h to infinity; C_{max}, peak plasma concentration; T_{max}, time of peak concentration; F, bioavailability; MAT, mean absorption time.

[a]Statistically different from IV.

[b]Statistically different from SC.

[c]Statistically different from PO.

[d]Median value.

different doses for the determined pharmacokinetic parameters in sheep was justified.

Our data indicated that RX has a moderate SC and low PO bioavailability, with mean values significantly different. Indeed, SC administration can evade drug metabolism (or hydrolysis) in the digestive tract, compared with oral administration (Benedetti et al., 2009). The reported bioavailability values were higher in fasted cats (69% SC; 49% PO; King et al., 2013) and dogs (88% SC; 84% PO fasted; 60% PO fed; Jung et al., 2009). A decrease in the rate of absorption in sheep can be associated with the abundant fermentation system by the ruminal microflora (Baggot & Brown, 1998), in addition to dilution and retention of the drug in the forestomach, compared with the diverse digestive system in monogastric species (Coetzee et al., 2011). Nevertheless, food is known to influence the absorption as well as binding of drugs reducing the total absorbed amount, especially for NSAIDs (Lees et al., 1998; Türck et al., 1996). It is also unknown whether RX binds to hay or digesta in ruminants, reducing furthermore bioavailability, which is the case for several NSAIDs such as phenylbutazone and flunixin meglumine (Lees et al., 1998). However, because most sheep will not have had food withheld in clinical settings, the results for the present study may reflect the pharmacokinetics of orally administered RX in a typical clinical setting. Although RX tablets provided either alone or with a minor amount of food might lead to a superior bioavailability (King et al., 2013), more studies are needed to settle this in sheep.

Accordingly, the dose-normalized $AUC_{(0-\infty)}$ of RX following IV administration was statistically higher than $AUC_{(0-\infty)}$ of the SC and PO routes, as lower fraction of the doses was absorbed in these two routes. As for MRT_{IV} which is significantly different from MRT_{SC} and MRT_{PO}, the longer residence time for the extravascular routes may be elucidated by the sustained time for absorption following SC and PO administrations (Albarellos et al., 2016).

In sheep (1.5 h), rats (1 h, King et al., 2009), dogs (0.5 h, Schmid, Spreng, et al., 2010; Borer et al., 2017), and cats (0.5 h; King et al., 2013), T_{max} was relatively short after oral administration. These data, alongside the relatively short half-life, are consistent with rapid absorption (Lees et al., 2022). Orally administered RX should be rapidly absorbed from the rumen, given its relatively high aqueous solubility of 0.17 g/L at a pH between 6.4 and 6.8. Moreover, its medium lipid solubility (log partition coefficient in n-octanol/phosphate buffer at pH 6.8 = 2.27) facilitates intestinal absorption (King et al., 2009).

In this study, the Vss following IV administration of RX at a dose of 2 mg/kg in sheep was low with 0.077 L/kg, and lesser than that previously reported in dogs (0.24 L/kg; Schmid, Spreng, et al., 2010), and in cats (0.19 L/kg; King et al., 2013). For NSAIDs generally, the low volume of distribution is associated to the very high plasma protein binding (King et al., 2009). The binding ratio of RX to plasma proteins is unknown in sheep. However, at a RX concentration of 2 µg/ml, protein binding exceeded 98% in dogs and cats (Jung et al., 2009). Furthermore, since the Vss value is similar to the sheep's blood volume which is 0.075 L/kg (Luethy et al., 2017), a plasma protein binding study would be also useful to assess whether the drug tends to remain in the extra- or intra-cellular compartment, since it

is an important factor to assess the drug efficacy (Lees et al., 2022). In fact, the selective distribution of RX to sites of inflammation has been demonstrated in rats, dogs, and cats and is attributable to its physicochemical property as a weak acid (pKa 4.7). A long residence time of RX in exudates was observed (>24 h), with a prolonged duration of action (King et al., 2009; Pelligand et al., 2012, 2014). This, however, would have to be also inspected in sheep, as it could have major clinical relevance.

In this study, the slow Cl (0.056 L/h/kg) of RX in sheep was slower than that previously reported in dogs (0.81 L/kg/h; Schmid, Spreng, et al., 2010) and cats (0.44 L/kg/h; King et al., 2013). The differences in Cl of RX between species can be attributed to variances in cardiac output. Indeed, the low estimated E for RX in sheep found in the present study (0.01) (Toutain & Bousquet-Mélou, 2004a, 2004b) was lower than that found in cats and dogs, for which the range was between 0.05 and 0.15 (King & Jung, 2021; classified as low to moderate; Toutain & Bousquet-Mélou, 2004a, 2004b). The lower ability to eliminate RX, compared with dogs, could be due to a lower hepatic extraction ratio, thus, the differences in the species isoform composition, expression, and activities of biotransformation enzymes. It could also be attributable to variances in renal clearance and its proportion (%) of overall clearance (Dantzler, 2016; Toutain & Bousquet-Mélou, 2004a, 2004b). Further studies to assess the routes of excretion and the metabolism of RX in sheep are needed.

The t1/2λz values were not statistically different for the three routes of administration, and longer than in cats (1.49 h; Schmid, Spreng, et al., 2010) and dogs (0.81 h; King et al., 2013). Despite the slow Cl, t1/2λz values were still relatively considered short. To note that, in dogs, RX has a longer duration (>24 h) of effect in illnesses involving peripheral inflammation due to its selectivity for inflammatory sites. This made RX suitable to be given to dogs once a day, despite what its short blood half-life would suggest (Lees et al., 2022). As previously stated, similar studies in diseased sheep are required to study this, because a possible prolonged duration of action, independently of t1/2λz, can considerably extend the dosage interval and lower the frequency of administration.

A limitation of this study is that no pharmacodynamic study was established. Circulating concentrations of NSAIDs required to provide good analgesia and anti-inflammatory effect should be of the order of the mean 80% inhibitory concentration (IC_{80}) value for COX-2 inhibition (Lees et al., 2004; Warner et al., 1999). The reported IC_{80} for COX-2 by RX was 0.1049 μg/ml in cats, and 0.163 μg/ml in dogs, and RX doses used in these studies provided analgesia. Regarding this study, in all sheep, RX concentrations were maintained above the mentioned IC_{80} of dogs for at least 10 h, for the three routes of administration. If it is assumed that sheep and dogs have a similar inhibitory concentration of COX-2, the doses experimentally tested in this study lead to plasma concentrations that might provide clinical effects (Giorgi et al., 2016; Sartini et al., 2021). This is also supported by the calculated mean AUC, which was at least five times higher in sheep than in dogs and cats (when doses normalized).

The PK/PD relationship for most of the analgesic or an anti-inflammatory drugs obeys to some indirect effects (Sharma & Jusko, 1998). However, the presence or not of a hysteresis effect in sheep is unknown and must be taken into consideration, despite that RX previously marked negative hysteresis in cats (Pelligand et al., 2012, 2014), explained by a distinct accumulation in the deep tissues, by a slow binding and release from the target receptor, and by a high potency for COX-2 inhibition in peripheral tissue (Pelligand et al., 2012).

Another limitation of this study lies in the study design. A crossover study would have been more suitable given that a parallel study would not have limited inter-individual variability. However, due to logistical difficulties when the study was being developed, it was not carried out. A final limitation would be the lack of assessment of a maximum residue limit (MRL) for food derivatives. Such findings are needed before widespread use of RX in sheep intended for human consumption. Even though that would limit the use of RX to experimental animals and sheep in wool production (Di Salvo et al., 2017), without tissue elimination data, one alternative for calculation of a preliminary withdrawal interval in food animal species is to multiply the terminal plasma t1/2λz by 10 (Riviere & Sundlof, 2009; Smith, 2013). Thus, a conservative meat withdrawal interval of 3 days may be suggested.

In conclusion, based on the observed findings, the SC route at 4 mg/kg seems to be the most convenient in terms of bioavailability compared with the PO single administration in sheep. RX deserves full consideration for further research on its efficacy and safety profile in sheep and, if applicable, on its tissue kinetics to establish a reliable withdrawal interval.

ACKNOWLEDGMENTS

None of the authors has any financial or personal relationships that could inappropriately influence or bias the content of this paper. This work was supported by University of Pisa (ex 60%). The authors acknowledge ThothPro (Gdansk, Poland) for supplying the software used for the pharmacokinetic analysis. Open Access Funding provided by Universita degli Studi di Sassari within the CRUI-CARE Agreement.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest in publishing this work.

DATA AVAILABILITY STATEMENT

The data sets used and/or analyzed during the current study are available from the corresponding author, upon reasonable request.

ETHICAL APPROVAL

The animal experiment was approved by the University of Lublin's animal welfare ethics committee and conducted in compliance with European law (Directive 2010/63/EU) on the protection of animals used for experimental and other scientific purposes.

ORCID

Charbel Fadel https://orcid.org/0000-0001-9996-5942
Beata Łebkowska-Wieruszewska https://orcid.org/0000-0002-1569-0599
Irene Sartini https://orcid.org/0000-0002-0538-4563
Andrzej Lisowski https://orcid.org/0000-0003-1463-9908
Amnart Poapolathep https://orcid.org/0000-0001-5322-3281
Mario Giorgi https://orcid.org/0000-0003-3657-4703

REFERENCES

Albarellos, G. A., Montoya, L., Lorenzini, P. M., Passini, S. M., Lupi, M. P., & Landoni, M. F. (2016). Pharmacokinetics of cefuroxime after intravenous, intramuscular, and subcutaneous administration to dogs. *Journal of Veterinary Pharmacology and Therapeutics*, *39*, 40–44.

Anonymous, 2012. Guideline on Bioanalytical Method Validation. Retrieved from: http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2011/08/WC500109686.pdf. Accessed 11 May 2022.

Anonymous, 2016. Retrieved from https://www.boehringer-ingelheim.com.au/sites/au/files/animal_health_new_zealand/metacam_20_nz.pdf. Accessed 07 July 2022.

Anonymous, 2020. Retrieved from https://www.agrovetmarket.com/Files/187c26f8-7427-402b-8ee1-20fba394e5b8.pdf. Accessed 07 July 2022.

Baggot, J. D., & Brown, S. A. (1998). Basis for selection of the dosage form. In G. E. Hardee & J. D. Baggot (Eds.), *Development and formulation of veterinary dosage forms* (2nd ed., pp. 7–143). Marcel Dekker Inc.

Benedetti, M. S., Whomsley, R., Poggesi, I., Cawello, W., Mathy, F. X., Delporte, M. L., Papeleu, P., & Watelet, J. B. (2009). Drug metabolism and pharmacokinetics. *Drug Metabolism Reviews*, *41*, 344–390.

Borer, L. R., Seewald, W., Peel, J. E., & King, J. N. (2017). Evaluation of the dose-response relationship of oral robenacoxib in urate crystal-induced acute stifle synovitis in dogs. *Journal of Veterinary Pharmacology and Therapeutics*, *40*, 148–157.

Coetzee, J. F., Mosher, R. A., Kohake, L. E., Cull, C. A., Kelly, L. L., Mueting, S. L., & KuKanich, B. (2011). Pharmacokinetics of oral gabapentin alone or co-administered with meloxicam in ruminant beef calves. *Veterinary Journal*, *190*, 98–102.

Coulter, C. A., Flecknell, P. A., & Richardson, C. A. (2009). Reported analgesic administration to rabbits, pigs, sheep, dogs and non-human primates undergoing experimental surgical procedures. *Laboratory Animals*, *43*, 232–238.

Dantzler, W. H. (2016). *Comparative physiology of the vertebrate kidney* (2nd ed.). Springer.

Di Salvo, A., Giorgi, M., Lee, H. K., Vercelli, C., Rueca, F., Marinucci, M. T., & Rocca, G. D. (2017). Plasma profile of cimicoxib in sheep after oral administration at two different rates. *Polish Journal of Veterinary Sciences*, *20*, 535–538.

European Medicines Agency. Onsior: European Public Assessment Report, Scientific discussion, (2008). Retrieved from: http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Scientific_Discussion/veterinary/000127/WC500067756.pdf. Accessed 30 Mar 2022.

Fadel, C., Sartini., I., & Giorgi., G. (2021). Paracetamol: A focus on dogs. *American Journal of Animal and Veterinary Sciences*, *16*, 247–262.

Flower, R. J. (2003). The development of COX2 inhibitors. *Nature Reviews. Drug Discovery*, *2*, 179–191.

Giorgi, M., De Vito, V., Poapolathep, A., Rychshanova, R., Sgorbini, M., & Owen, H. (2016). Pharmacokinetics and disposition of flupirtine in the horse. *Veterinary Journal*, *208*, 76–80.

Huxley, J. N., & Whay, H. R. (2007). Attitudes of UK veterinary surgeons and cattle farmers to pain and the use of analgesics in cattle. *Cattle Practice*, *15*, 189–193.

Lizarraga, I., & Chambers, J. P. (2012). Use of analgesic drugs for pain management in sheep. *New Zealand Veterinary Journal*, *60*, 87–94.

Julious, S. A., & Debarnot, C. A. (2000). Why are pharmacokinetic data summarized by arithmetic means? *Journal of Biopharmaceutical Statistics*, *1*, 55–71.

Jung, M., Lees, P., Seewald, W., & King, J. N. (2009). Analytical determination and pharmacokinetics of robenacoxib in the dog. *Journal of Veterinary Pharmacology and Therapeutics*, *32*, 41–48.

King, J. N., Dawson, J., Esser, R. E., Fujimoto, R., Kimble, E. F., Maniara, W., Marshall, P. J., O'Byrne, L., Quadros, E., Toutain, P. L., & Lees, P. (2009). Preclinical pharmacology of robenacoxib: A novel selective inhibitor of cyclooxygenase-2. *Journal of Veterinary Pharmacology and Therapeutics*, *32*, 1–17.

King, J. N., Jung, M., Maurer, M. P., Schmid, V. B., Seewald, W., & Lees, P. (2013). Effects of route of administration and feeding schedule on pharmacokinetics of robenacoxib in cats. *American Journal of Veterinary Research*, *74*, 465–472.

King, J. N., Rudaz, C., Borer, L., Jung, M., Seewald, W., & Lees, P. (2011). In vitro and ex vivo inhibition of canine cyclooxygenase isoforms by robenacoxib: A comparative study. *Research in Veterinary Science*, *88*, 497–506.

King, J. N., & Jung, M. (2021). Determination of the route of excretion of robenacoxib (Onsior™) in cats and dogs: A pilot study. *Journal of Veterinary Pharmacology and Therapeutics*, *44*, 411–416.

Lees, P., Toutain, P. L., Elliott, J., Giraudel, J. M., Pelligand, L., & King, J. N. (2022). Pharmacology, safety, efficacy and clinical uses of the COX-2 inhibitor robenacoxib. *Journal of Veterinary Pharmacology and Therapeutics*, *45*(4), 325–351.

Lees, P., Landoni, M. F., Giraudel, J. M., & Toutain, P. L. (2004). Pharmacodynamics and pharmacokinetics of non-steroidal anti-inflammatory drugs in species of veterinary interest. *Journal of Veterinary Pharmacology and Therapeutics*, *27*, 479–490.

Lees, P., McKellar, Q. A., Foot, R., & Gettinby, G. (1998). Pharmacodynamics and pharmacokinetics of tolfenamic acid in ruminating calves: Evaluation in models of acute inflammation. *Veterinary Journal*, *155*, 275–288.

Pairet, M., & Engelhardt, G. (1996). Distinct isoforms (COX-1 and COX-2) of cyclooxygenase: Possible physiological and therapeutic implications. *Fundamental & Clinical Pharmacology*, *10*, 1–17.

Pelligand, L., King, J. N., Hormazabal, V., Toutain, P. L., Elliott, J., & Lees, P. (2014). Differential pharmacokinetics and pharmacokinetic/pharmacodynamic modelling of robenacoxib and ketoprofen in a feline model of inflammation. *Journal of Veterinary Pharmacology and Therapeutics*, *37*, 354–366.

Pelligand, L., King, J. N., Toutain, P. L., Elliott, J., & Lees, P. (2012). Pharmacokinetic/pharmacodynamic modelling of robenacoxib in a feline tissue cage model of inflammation: Robenacoxib PK/PD modelling in the cat. *Journal of Veterinary Pharmacology and Therapeutics*, *35*, 19–32.

Riviere, J. E., & Sundlof, S. F. (2009). Chemical residues in tissues of food animals. *Journal of Veterinary Pharmacology and Therapeutics*, *7*, 1148–1157.

Sartini, I., Łebkowska-Wieruszewska, B., Lisowski, A., Poapolathep, A., Cuniberti, B., & Giorgi, M. (2021). Pharmacokinetics of acetaminophen after intravenous and oral administration in fasted and fed Labrador retriever dogs. *Journal of Veterinary Pharmacology and Therapeutics*, *44*, 28–35.

Schmid, V. B., Seewald, W., Lees, P., & King, J. N. (2010). In vitro and ex vivo inhibition of COX isoforms by robenacoxib in the cat: A comparative study. *Journal of Veterinary Pharmacology and Therapeutics*, *33*, 444–452.

Schmid, V. B., Spreng, D. E., Seewald, W., Jung, M., Lees, P., & King, J. N. (2010). Analgesic and anti-inflammatory actions of robenacoxib in acute joint inflammation in dog. *Journal of Veterinary Pharmacology and Therapeutics*, *33*, 118–131.

Sharma, A., & Jusko, W. J. (1998). Characteristics of indirect pharmacodynamic models and applications to clinical drug responses. *British Journal of Clinical Pharmacology*, *45*, 229–239.

Small, A. H., Belson, S., Holm, M., & Colditz, I. G. (2014). Efficacy of a buccal meloxicam formulation for pain relief in merino lambs undergoing knife castration and tail docking in a randomised field trial. *Australian Veterinary Journal*, *92*, 381–388.

Smith, G. (2013). Extralabel use of anesthetic and analgesic compounds in cattle. Vet. *Clinics of North America: Food Animal Practice*, *29*, 29–45.

Smith, J. S., Schleining, J., & Plummer, P. (2021). Pain Management in Small Ruminants and Camelids: Analgesic agents. Vet. *Clinics of North America: Food Animal Practice*, *37*, 1–16.

Steagall, P. V., Bustamante, H., Johnson, C. B., & Turner, P. V. (2021). Pain Management in Farm Animals: Focus on cattle, Sheep and Pigs. *Animals*, *11*, 1483.

Stewart, M., Verkerk, G. A., Stafford, K. J., Schaefer, A. L., & Webster, J. R. (2010). Noninvasive assessment of autonomic activity for evaluation of pain in calves, using surgical castration as a model. *Journal of Dairy Science*, *93*, 3602–3609.

Stuart, A. K., KuKanich, B., Caixeta, L. S., Coetzee, J. F., & Barrell, E. A. (2019). Pharmacokinetics and bioavailability of oral firocoxib in adult, mixed-breed goats. *Journal of Veterinary Pharmacology and Therapeutics*, *42*, 640–646.

Toutain, P. L., & Bousquet-Mélou, A. (2004a). Plasma terminal half-life. *Journal of Veterinary Pharmacology and Therapeutics*, *27*, 427–439.

Toutain, P. L., & Bousquet-Mélou, A. (2004b). Plasma clearance. *Journal of Veterinary Pharmacology and Therapeutics*, *27*, 415–425.

Türck, D., Roth, W., & Busch, U. (1996). A review of the clinical pharmacokinetics of meloxicam. *British Journal of Rheumatology*, *35*, 6–13.

Turk, E., Tekeli, I. O., Durna Corum, D., Corum, O., Altinok Yipel, F., Ilhan, A., Emiroglu, S. B., Uguz, H., & Uney, K. (2021). Pharmacokinetics of tolfenamic acid in goats after different administration routes. *Journal of Veterinary Pharmacology and Therapeutics*, *44*, 367–373.

Luethy, D., Stefanovski, D., Salber, R., & Sweeney, R. W. (2017). Prediction of packed cell volume after whole blood transfusion in Small ruminants and south American camelids: 80 cases (2006-2016). *Journal of Veterinary Internal Medicine*, *31*, 1900–1904.

Warner, T. D., Giuliano, F., Vojnovic, I., Bukasa, A., Mitchell, J. A., & Vane, J. R. (1999). Nonsteroid drug selectivities for cyclooxygenase-1 rather than cyclooxygenase-2 are associated with human gastrointestinal toxicity: A full in vitro analysis. *Proceedings of the National Academy of Sciences of the United States of America*, *96*, 7563–7568.

Welsh, E. M., & Nolan, A. M. (1995). Effect of flunixin meglumine on the thresholds to mechanical stimulation in healthy and lame sheep. *Research in Veterinary Science*, *58*, 61–66.

How to cite this article: Fadel, C., Łebkowska-Wieruszewska, B., Sartini, I., Lisowski, A., Poapolathep, A., & Giorgi, M. (2022). Robenacoxib pharmacokinetics in sheep following oral, subcutaneous, and intravenous administration. *Journal of Veterinary Pharmacology and Therapeutics*, *00*, 1–8. https://doi.org/10.1111/jvp.13089