In vitro and ex vivo inhibition of COX isoforms by robenacoxib in the cat: a comparative study

V. B. SCHMID* W. SEEWALD† P. LEES‡ & J. N. KING†

*Novartis Centre de Recherche Santé Animale SA, St-Aubin, Switzerland; †Novartis Animal Health Inc., Basel, Switzerland; ‡Royal Veterinary College, Hawkshead Campus, Hatfield, Hertfordshire, UK

INTRODUCTION

Nonsteroidal anti-inflammatory drugs (NSAIDs) are widely used in veterinary and human medicine for their analgesic, anti-inflammatory and anti-pyretic actions. In comparison with dogs, relatively few NSAIDs are registered for use in cats (carprofen, ketoprofen, meloxicam and tolifenamic acid), partially because of safety issues with some of the drugs of this class in this species (Lascelles et al., 2007). However there is increasing recognition of a major requirement for effective and safe analgesics for therapeutic use in the cat (Robertson & Taylor, 2004; Taylor & Robertson, 2004; Robertson, 2005; Lascelles et al., 2007).

In recent years, newer NSAIDs of the coxib class have become available. These drugs are selective inhibitors of the COX-2 isoform and may offer safety advantages over nonselective inhibitors in causing less damage to the gastrointestinal tract (Watson et al., 2000; Schnitzer et al., 2004) and not interfering with blood clotting (Masferrer et al., 1994; Warner et al., 1999). The safety benefits of COX-2 selective NSAIDs have been established in humans (Schnitzer et al., 2004) and rats (King et al., 2009), but data in cats are at present lacking. Two coxib NSAIDs, namely deracoxib and firocoxib, have been registered for use in dogs but not in cats (Gierse et al., 2002; Millis et al., 2002; McCann et al., 2004; Sessions et al., 2005; Hanson et al., 2006; Pollmeier et al., 2006).

Robenacoxib is a novel nonsteroidal anti-inflammatory drug (NSAID) developed for use in companion animals. Whole blood assays were used to characterize in the cat the pharmacodynamics of robenacoxib for inhibition of the cyclooxygenase (COX) isoforms, COX-1 and COX-2, in comparison with other NSAIDs. Based on in vitro IC₅₀ COX-1:IC₅₀ COX-2 ratios, robenacoxib was COX-2 selective (ratio = 32.2), diclofenac (ratio = 3.9) and meloxicam (ratio = 2.7) were only weakly COX-2 preferential, and ketoprofen (ratio = 0.049) was COX-1 selective. In an in vivo pharmacokinetic ex vivo pharmacodynamic study, after both p.o. (1–2 mg/kg) and subcutaneous (2 mg/kg) dosing, robenacoxib achieved peak blood concentrations rapidly (Tₘₚₓₛ = 1 h for both administration routes) and was cleared from blood relatively rapidly (mean residence time was 1.70 h after p.o. and 1.79 h after subcutaneous dosing). In ex vivo COX isoform inhibition assays, orally (1–2 mg/kg) or subcutaneously (2 mg/kg) administered robenacoxib significantly inhibited COX-2, with a relatively short duration of action in the central compartment, and had no effect on COX-1. Therefore robenacoxib was COX-2 selective and spared COX-1 in vivo. In contrast, meloxicam (0.3 mg/kg via subcutaneous injection) inhibited both COX-1 and COX-2 isoforms significantly for at least 24 h, indicating nonselectivity in vivo.

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V. B. Schmid, Novartis Centre de Recherche Santé Animale SA, CH-1566 St-Aubin, Switzerland. E-mail: vincent.schmid@novartis.com

Robenacoxib is a novel coxib NSAID which has been developed exclusively for veterinary use (King et al., 2009). It is an analogue of diclofenac, a preferential inhibitor of COX-2. Recent studies have established that robenacoxib is a highly selective inhibitor of COX-2 in the rat, dog and cat (Giraudel et al., 2009; King et al., 2009, 2010). The effect of robenacoxib on COX-1 and COX-2 in cats in vitro, compared with meloxicam and caprofen, was reported recently by Giraudel et al. (2009).

The aim of this study was to extend the work of Giraudel et al. (2009) to further evaluate the effect of robenacoxib on COX-1 and COX-2 in cats. Whole blood in vitro assays for COX-1 and COX-2 were used, as these are considered to be the most relevant to NSAID actions in vivo (Patrignani et al., 1994; Pairet & Engelhardt, 1996; Giraudel et al., 2005). In contrast to the study with robenacoxib by Giraudel et al. (2009), we used the conventional lipopolysaccharide (LPS) stimulated production of PGE₂ for the COX-2 assay. First, the effects of robenacoxib on
COX-1 and COX-2 in vitro were compared with those of two other NSAIDs licensed for feline use (ketoprofen and meloxicam) and with the reference COX-2 preferential drug diclofenac. Second, the effect of robenacoxib, administered p.o. and subcutaneously at the registered dosages, on COX-1 and COX-2 were evaluated in an in vivo pharmacokinetic/ex vivo pharmacodynamic study. The effects of robenacoxib in that study were compared to both a negative control and a positive control (meloxicam administered subcutaneously).

The hypothesis of the study was that robenacoxib would be selective for COX-2 in vitro, and in consequence would inhibit COX-2 and spare COX-1 when administered at the recommended dosages to cats.

MATERIALS AND METHODS

The studies were conducted under protocols approved by the Novartis Animal Health Research Centre in St-Aubin, Switzerland, and in compliance with the Swiss law for animal protection.

In vitro studies

COX-1 and COX-2 assays
Concentration-effect relationships for inhibition of COX-1 and COX-2 in feline whole blood assays were established for robenacoxib in comparison with three control drugs. Diclofenac was selected as a drug of similar chemical structure to robenacoxib; which, in previous studies in dogs, rats and humans was nonselective or slightly preferentially selective for COX-2 (King et al., 2009, 2010). The other comparator drugs, ketoprofen and meloxicam, were chosen because both are licensed for use in cats. Each drug was supplied as a dry powder from Sigma-Aldrich Chemie GmbH, Buchs, Switzerland (diclofenac, racemic ketoprofen and meloxicam) and CarboGen AG, Aarau, Switzerland (robenacoxib). Because of the large number of samples for analysis, the study was conducted in two phases: robenacoxib and diclofenac in part 1 and ketoprofen and meloxicam in part 2.

Blood was collected from eight healthy cats (four male and four female) on four occasions. For the COX-1 assay, a volume of 5 mL blood was collected by venepuncture into plastic tubes without anticoagulant (S-Monovette®, Sarstedt AG, Sevelen, Switzerland). Aliquots of 0.25 mL blood were pipetted into microtubes containing a range of concentrations of one of the four drugs dissolved in 20 µL DMSO, followed by 5 µL LPS obtained from Sigma-Aldrich GmbH, Buchs, Switzerland (L-2630 E. coli serotype O111:B4). The LPS was dissolved in sterile phosphate buffered saline (PBS, Sigma-Aldrich GmbH, Buchs, Switzerland, S-8776) at a concentration of 5.04 mg/mL, divided into aliquots and frozen at −20 °C. A fresh aliquot was used in each assay. A total of 15 doubling concentrations was used for each drug over the following concentration ranges: robenacoxib and diclofenac 0.0006–10 µM; ketoprofen 0.018–3000 µM and meloxicam 0.006–100 µM. After vortexing, tubes were incubated for 24 h at 37 °C, and then centrifuged at 16 000 g at 4 °C for 10 min. The supernatant plasma was stored at −20 °C for not more than 1 week before assay for PGE2 by enzyme immunoassay using commercial kits (Amersham Pharmacia, RPN220-PGE2, Biotrak EIA, GE Healthcare GmbH, Otelfingen, Switzerland). Plasma samples were processed according to the manufacturer’s indications (a separate standard curve was run for each microplate; standards and samples were assayed in duplicate; duplicates showing a CV > 15% were re-analyzed).

Curve fitting and data analysis

All calculations were undertaken using SAS®, Version 8.2 software (SAS Institute Inc., Cary, NC, USA, 1999).

For both assays the concentration-response data were fitted to a standard sigmoidal model using SAS® Proc Nlin:

\[
y = y_1 + \frac{y_0 - y_1}{1 + \exp(x + \beta \log x)}
\]

where \(x\) = drug concentration, \(y\) = response with the following parameters. \(x = \text{intercept}, \ \beta = \text{slope} \ (>0), \ y_0 = \text{y value for} \ x = 0, \ y_1 = \text{y value for} \ x = \infty\), \ IC_{50} = \text{the value of} \ x \ \text{for which} \ y = (y_0 + y_1)/2, \ \text{therefore} \ IC_{50} = \exp(-a/\beta)

As the model is nonlinear, the estimation of parameters involves an iterative technique. Estimates of model uncertainty were then based on linear approximations. It was assumed that \(y_1\) is zero.

Initially, an attempt was made to fit the data for one response, one drug and one cat. However, fits were poor in all cases. Therefore in the final model, the data were fitted to one response, one drug and all animals with parameters \(x\) and \(y_0\) varying between cats but \(\beta\) being the common slope for all cats.

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Geometric means were calculated for all cats and geometric mean ratios determined, as follows: IC\textsubscript{50}COX-1:IC\textsubscript{50}COX-2, IC\textsubscript{80}COX-1:IC\textsubscript{80}COX-2, IC\textsubscript{20}COX-1:IC\textsubscript{20}COX-2. In addition, to further characterize selectivity for COX-2, the magnitude of TxB\textsubscript{2} inhibition was determined as a function of PGE\textsubscript{2} inhibition.

In vivo/ex vivo study

Animals and study design

An in vivo pharmacokinetic/ex vivo pharmacodynamic study was undertaken under GLP compliant conditions. The main objective was to determine the time course of inhibition of production of serum TxB\textsubscript{2} and plasma PGE\textsubscript{2} using whole blood assays. A secondary objective was to establish blood concentration–time profiles and derive pharmacokinetic variables. Eight cats (four male, four female) of the domestic short-hair breed were used, aged 2 years 11 months to 4 years 3 months and weighing 2.5–6 kg. The cats were fed a dry pelleted feed once daily, when group housed. For 2 days before and 24 h after each drug administration, the cats were housed individually and fed a mix of dry and wet food. Water was available ad libitum.

The cats were randomly allocated to receive each of four treatments in sequence in a four-part Latin Square design with an interval of approximately 2 weeks between phases. The four treatments administered were:

- a single tablet or fractions of a tablet of robenacoxib administered orally at a dose of 1–2 mg/kg using tablets containing 6 mg robenacoxib (Novartis Santé Animale S.A.S., Hunningue, Cedex, France).
- robenacoxib injection administered subcutaneously at a dose of 2 mg/kg (2% w/v solution for injection, Vericore Ltd., Dundee, Scotland).
- as a positive control meloxicam injection (Metacam\textsuperscript{®}; 5 mg/mL, Labiana Life Sciences S.A., Terrassa, Spain) administered subcutaneously at a dose of 0.3 mg/kg.
- a negative control group (cats sham dosed by injection or orally).

Subcutaneous injections of robenacoxib and meloxicam were administered in the scruff of the neck using exact doses based on body weight and achieved by weighing syringes. For oral dosing of robenacoxib, cats weighing 3–6 kg received one entire 6 mg tablet. Cats weighing less than 3 kg were given a fraction of a tablet, to achieve a dose close to 2 mg/kg, using a tablet splitter to achieve halving and quartering of tablets. Tablets or fractions of tablets were placed on the pharynx behind the tongue, followed by a small quantity of water to ensure ingestion. Tablets or fractions of tablets were weighed prior to administration. Cats were fasted overnight and then provided with food approximately 2 h after dosing.

Blood samples

Blood samples (3.6 mL) were collected before and at 1, 2, 4, 6, 8 and 24 h after dosing in order to: (i) measure blood concentrations of robenacoxib; and (ii) determine the magnitude and time course of inhibition of COX-1 and COX-2 ex vivo in whole blood assays. Samples were collected by direct puncture from a left or right jugular or cephalic vein into S-monovette\textsuperscript{®} tubes (Sarstedt AG, Sevelen, Switzerland) containing: (i) EDTA as anticoagulant (1.2 mL) for measurement of robenacoxib concentration; (ii) sodium citrate as anticoagulant (1.4 mL) for estimation of COX-2 activity; and (iii) without anticoagulant (1.0 mL) for determination of COX-1 activity. COX-1 and COX-2 activities were determined using the same enzyme immunoassays as described for the in vitro assays (vide supra).

Although a total of approximately 25 mL of blood (comprising an estimated 5–15% of total blood volume) was taken from the cats over 24 h, the blood was not replaced. Repeated puncture of different veins was preferred over catheterization to avoid the need for heparin administration or the risk of coagulation in the catheter.

Robenacoxib analysis

Blood samples were analyzed for robenacoxib using the methods described by Jung et al. (2009); an initial HPLC-UV method covering the range of approximately 500–20 000 ng/mL and, if required, a subsequent analysis by LC-MS covering the range of approximately 3–100 ng/mL. Depending on the results obtained with the UV method, specimens were diluted in order not to exceed 100 ng/mL in the MS method.

The analytical method was validated using as quality controls (QCs) spiked matrix specimens at concentrations of 5, 20 and 50 ng/mL, run with each sequence of unknown samples and independent of calibration standards. The assay met the requirement that two-thirds of QC samples are encompassed within ±15% (or ±20% for the lower limit of quantitation) of spiked concentrations. Samples containing robenacoxib in blood were shown to be stable for 5 months at −20 °C. All samples were analyzed within 10 weeks of collection. The lower limit of quantitation of the analytical method was 3 ng/mL.

Pharmacokinetics

The following pharmacokinetic variables were determined from the individual blood robenacoxib profiles after each administration:

- Area under the curve: \(AUC_{(0\text{–}t)}\) and \(AUC_{(0\text{–}\infty)}\)
- Area under the moment curve: \(AUMC_{(0\text{–}\infty)}\)
- Time of maximum blood concentration: \(T_{\text{max}}\)
- Maximum blood concentration: \(C_{\text{max}}\)
- Terminal half-life: \(t_{1/2}\)
- Mean residence time: \(MRT\)

The data were processed as individual time vs. blood concentration profiles using a validated pharmacokinetic evaluation software (DEBA, Data Evaluation in BioAnalytics, release 4.1 XP, Copyright Dr C. N. Thumm-Kraus, Software and Consulting, 1999, Basel, Switzerland). Mean values and SDs of pharmaco-
kinetic variables, dose normalized (where applicable) and mean blood concentration profiles were calculated using EXCEL.

Statistical evaluation

Data are presented as arithmetic mean, geometric mean or median plus SD or the 95% CI. Comparisons between different groups for PGE2 and TxB2 concentrations (as indices of COX-2 and COX-1 activity, respectively) were made statistically using repeated measures analysis of covariance (RM ANOVA) with the following effects in the model: phase, sequence, sex, baseline value, treatment group, time and treatment × time interaction. In the event of overall significance, post-hoc comparisons were made using linear contrasts. P values less than 0.05 were considered to be statistically significant.

RESULTS

In vitro study

The robenacoxib concentration–response relationships for inhibition of plasma PGE2, as an index of COX-2, and serum TxB2, as an index of COX-1, are shown in Figs 1 and 2, respectively. Geometric mean values and 95% CI for three levels of inhibition of COX-2 and COX-1; IC20, IC50 and IC80, and slope of the concentration–response relationships are presented in Table 1.

Slopes were relatively shallow and for each drug they were less steep for TxB2 than for PGE2 inhibition and this was significant for robenacoxib and meloxicam. Based on the three reported levels of inhibition, rank order of potency was diclofenac > robenacoxib > ketoprofen & meloxicam for COX-2 and ketoprofen > diclofenac > meloxicam > robenacoxib for COX-1. Based on IC50 values, robenacoxib was selective for COX-2, diclofenac and meloxicam were only slightly preferential for COX-2 and ketoprofen was selective for COX-1 (Table 2).

For diclofenac and ketoprofen, inhibition ratios were independent of level of inhibition, whereas the selectivity of robenacoxib and meloxicam increased from IC20TxB2:IC20PGE2 to IC80TxB2:IC80PGE2 (Table 2). The selectivities of robenacoxib and ketoprofen for inhibition of COX-1 and COX-2, respectively, were further confirmed by the IC20TxB2:IC80PGE2 ratios of 4.233 (robenacoxib) and 0.0052 (ketoprofen).

Data in Table 3 present percentage inhibition of COX-1 for given levels of COX-2 inhibition. The calculated values depend both on the distance between the COX-1 and COX-2 inhibition curves and curve slopes. Ninety five percent inhibition of COX-2 by robenacoxib produced only 12.4% COX-1 inhibition, whereas meloxicam inhibited COX-1 by 72.7% for 95% COX-2 inhibition. In contrast, 50% COX-2 inhibition by ketoprofen was associated with 97.7% COX-1 inhibition. Figure 3 illustrates the percentage inhibition of TxB2 versus percentage inhibition of COX-1 for robenacoxib and the three comparator drugs.

In vivo/ex vivo study

Eicosanoid inhibition

The time courses of ex vivo inhibition of plasma PGE2 and serum TxB2 are illustrated in Figs 4 and 5, respectively.

In the control group, eicosanoid concentrations showed some variability with time, but with no obvious increasing or decreasing trends.
Meloxicam significantly inhibited plasma PGE\textsubscript{2} concentration relative to the control treatment at all sampling times (Fig. 5, Table 4). Robenacoxib also inhibited PGE\textsubscript{2} and differences from control were marked but relatively transient; differences were significant for 2 h after p.o. dosing and for 6 h after subcutaneous administration (Table 4). The median times to maximal inhibition of plasma PGE\textsubscript{2} were: 2 h robenacoxib injection, 1 h robenacoxib tablets and 2 h meloxicam injection.

Meloxicam inhibited TxB\textsubscript{2} synthesis relative to control treatment at all times between 1 and 24 h, except for the 4 h sample when the decrease ($P = 0.0583$) was not significant (Fig. 5, Table 4). In addition, meloxicam produced significantly greater and more persistent inhibition of serum TxB\textsubscript{2} than the two robenacoxib groups (Table 4).

Neither oral nor parenteral dosing with robenacoxib significantly altered serum TxB\textsubscript{2}, except for a significant effect at 24 h after oral administration (Fig. 5, Table 4). The latter result is judged unlikely to be a true biological effect, since robenacoxib was no longer detected in the blood after 8 h. At several sampling times, serum TxB\textsubscript{2} was significantly lower after meloxicam than with either robenacoxib treatment (Table 4).

**Robenacoxib pharmacokinetics**

The robenacoxib concentration-time profile in blood is presented in Fig. 6. Higher and more persistent concentrations were obtained after subcutaneous dosing. Thus, arithmetic mean ± SD concentrations at 1 h were $1308 ± 481$ ng/mL (subcutaneous)
and 856 ± 639 (p.o.). Corresponding values at 4 h were 156 ± 158 and 20 ± 11 ng/mL. Mean dosages were 2.0 ± 0.0 mg/kg (oral) and 1.45 ± 0.34 mg/kg (p.o.).

After subcutaneous administration of robenacoxib the median value of $T_{\text{max}}$ was 1 h. Geometric mean mean residence time (MRT) was 1.79 h and terminal half-life was 0.97 h. Geometric mean values of $C_{\text{max}}$ and $AUC_{(0\text{-inf})}$, normalized to a dosage of 1 mg/kg, were 694 ng/mL and 1431 ng/mLh, respectively (Table 5).

After oral dosing of robenacoxib the median $T_{\text{max}}$ value was 1.0 h, geometric mean MRT was 1.70 h and terminal half-life was 1.53 h, somewhat longer than after subcutaneous administration. Geometric mean values of $C_{\text{max}}$ and $AUC$, normalized for a dose of 1.0 mg/kg, were both lower than after subcutaneous administration. For all variables inter-animal variability was greater after oral than subcutaneous dosing, as indicated by CV% values (Table 5).

**DISCUSSION**

The results of this study confirm the findings of Giraudel et al. (2009) that robenacoxib is a selective inhibitor of COX-2 in vitro in cats. In addition, using ex vivo assays, the recommended dosages of robenacoxib (2 mg/kg by injection, 1–2 mg/kg by tablets) produced significant inhibition of COX-2 while sparing COX-1.

The scientific literature contains many reports of in vitro studies undertaken to determine the potency of a wide range of NSAIDs as inhibitors of COX isoforms. As discussed by Warner et al. (1999) and Lees et al. (2004), assay results are markedly dependent on experimental conditions, so that isolated enzymes, broken cell preparations and intact cell assays, all in buffer solutions, and intact cell whole blood assays have yielded very disparate findings. Most investigators conducting in vitro assays have preferred to use whole blood, on the ground of greatest physiological relevance: such assays use a matrix which is a natural body fluid containing both platelets and leucocytes and in which the very high degree of drug binding to plasma protein, which characterises the great majority of NSAIDs, is allowed for (Young et al., 1996; Pairet & Van Ryn, 1998). However, even optimized in vitro blood assays can yield for individual drugs differing ratios to those obtained ex vivo and in vivo. This occurs, for example, for those drugs such as aspirin, phenylbutazone and tepoxalin, which are converted in vivo to active metabolites (Lees et al., 2004). Therefore, optimal assessment of NSAID effects on COX-1 and COX-2 should be based on results obtained from more than one method, as in the present investigation.

The present findings confirm previous results from Giraudel et al. (2009), demonstrating the selectivity of robenacoxib for COX-2 compared to COX-1 inhibition in in vitro whole blood assays. Further, the ex vivo data demonstrate marked inhibition of COX-2 with no inhibition of COX-1 at the recommended dosages of robenacoxib (1–2 mg/kg orally and 2 mg/kg via subcutaneous injection). This result indicates the likely absence of metabolism of robenacoxib in vivo to active metabolite(s), in relevant amounts, which possess a different pharmacodynamic profile to robenacoxib.

It is not possible to state precisely the level and duration of COX-2 inhibition which should be achieved to provide appropriate levels of analgesia or inhibition of inflammation or fever. Equally, the threshold in terms of magnitude and duration of inhibition of COX-1, which should not be exceeded in order to minimise the potential for side-effects on the gastrointestinal tract and impairment of haemostatic mechanisms, is unknown. However, COX-1 inhibition should ideally be as short as possible and not exceed 10–20% at peak concentration (Warner et al., 1999; Lees et al., 2004). In contrast, therapeutic dosages of most
NSAIDs in humans inhibit COX-2 by more than 50% and many are associated with 74–89% inhibition over the dosage interval (Warner et al., 1999). Lees (2003) proposed a similar level of inhibition for NSAIDs in animals. Based on these tentative criteria, the fact that robenacoxib produced only 4.4% COX-1 inhibition at a concentration that inhibited COX-2 by 80% in the in vitro whole blood assays may be regarded as an indicator of a probable good safety profile in vivo. The wide safety margin of robenacoxib for gastrointestinal, haemostatic and renal effects has been confirmed more directly in safety studies in the rat (King et al., 2009), dog and cat [King et al., 2010 (data on file)].

In contrast, the three comparator drugs used in this study produced much greater COX-1 inhibition for an 80% level of COX-2 inhibition. The findings with ketoprofen and meloxicam are of particular interest because both are licensed for clinical use in the cat. The in vitro blood assays demonstrated that ketoprofen is COX-1 selective in the cat; the concentration required for 50% COX-2 inhibition produced 98% inhibition of COX-1. In the light of this observation it is of further interest to note that ketoprofen, when administered p.o. and intravenously to cats at recommended dosages, produced profound and long lasting inhibition of COX-1 ex vivo, when assessed in whole blood assays (Lees et al., 2003). However, ketoprofen is a chiral compound that undergoes inversion of the R to the S isomer in vivo (Lees et al., 2003). S-ketoprofen is the eutomer, i.e. the enantiomer.

**Table 4.** Statistical comparison (by RMANCOVA followed by linear contrasts for post-hoc comparisons) of ex vivo plasma PGE2 and serum TxB2 concentrations after four treatments

<table>
<thead>
<tr>
<th>Eicosanoid</th>
<th>Time (h)</th>
<th>I vs. T</th>
<th>I vs. M</th>
<th>T vs. M</th>
<th>I vs. C</th>
<th>T vs. C</th>
<th>M vs. C</th>
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<td>T &lt;.0001</td>
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<td></td>
<td>2</td>
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<td>0.2265</td>
<td>0.8116</td>
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<td>I &lt;.0001</td>
<td>T &lt;.0001</td>
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<td></td>
<td>4</td>
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<td>I 0.2684</td>
<td>0.0001</td>
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<td>M 0.2288</td>
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<td></td>
<td>24</td>
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<td>0.0014</td>
<td>I 0.0008</td>
<td>M 0.4914</td>
<td>I 0.5043</td>
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<td>TxB2</td>
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<td>T 0.0159</td>
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</table>

* = robenacoxib injection administered subcutaneously, T = robenacoxib tablet administered p.o., M = meloxicam injection administered subcutaneously, C = control (no treatment). Data from eight cats. Next to each significant comparison (bold type), the group with the lower concentration of the eicosanoid is indicated.

**Table 5.** Pharmacokinetics of robenacoxib after single oral (1–2 mg/kg) and subcutaneous (2 mg/kg) administration to eight cats

<table>
<thead>
<tr>
<th>Variable (units)</th>
<th>Dosing (p.o.)</th>
<th>Dosing (subcutaneous)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Geometric mean</td>
<td>Arithmetic mean</td>
</tr>
<tr>
<td>T&lt;sub&gt;max&lt;/sub&gt; (h)</td>
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<td></td>
</tr>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt; norm. (ng/mL)&lt;sup&gt;3&lt;/sup&gt;</td>
<td>493</td>
<td>626</td>
</tr>
<tr>
<td>AUC&lt;sub&gt;0–t&lt;/sub&gt; norm. (ng/mL·h)&lt;sup&gt;3&lt;/sup&gt;</td>
<td>694</td>
<td>858</td>
</tr>
<tr>
<td>AUC&lt;sub&gt;0–inf&lt;/sub&gt; norm. (ng/mL·h)&lt;sup&gt;3&lt;/sup&gt;</td>
<td>719</td>
<td>878</td>
</tr>
<tr>
<td>MRT (h)</td>
<td>1.70</td>
<td>1.87</td>
</tr>
<tr>
<td>Terminal t&lt;sub&gt;1/2&lt;/sub&gt; (h)</td>
<td>1.53</td>
<td>1.95</td>
</tr>
</tbody>
</table>

*Pharmacokinetic calculations performed with the software DEBA. AUC was calculated by linear trapezoidal integration. <sup>3</sup>For T<sub>max</sub> there was no Gaussian distribution; therefore median values are presented. <sup>3</sup>Normalized to a dosage of 1 mg/kg for all animals.
with greatest potency. Therefore in vitro and in vivo effects of ketoprofen on COX-1 and COX-2 may be expected to differ. Also to be noted is the possibility of species variation in the selectivity of ketoprofen for COX isoforms. Using the same in vitro whole blood assays as those used in the present investigation, King et al. (2010) reported for ketoprofen in the dog an IC50 COX-1:IC50 COX-2 ratio of 0.88, suggesting that it is a nonselective COX inhibitor in this species.

The present results confirm previous work that meloxicam has low selectivity for COX-2 in vitro in cats (Giraudel et al., 2009). The low COX-2 selectivity observed in vitro translated into a lack of selectivity in vivo, with significant inhibition of both COX-1 and COX-2 with the 0.3 mg/kg dosage with subcutaneous injection.

In view of possible inter-species differences and differences attributable to experimental technique, even when using whole blood as the matrix, it is relevant to compare for robenacoxib COX inhibition potencies and potency ratios in several species, including those of intended clinical use, the cat and dog. For the dog (King et al., 2010) and the cat (this study). IC50 values for COX-1 inhibition, respectively, were 10.77 and 4.98 μM. Corresponding values for COX-2 inhibition were 0.079 and 0.111 μM and IC50 COX-1:IC50 COX-2 inhibition ratios were 129:1 and 32:1. Also using whole blood assays, but with a different methodology from the present investigation, Giraudel et al. (2009) reported the following values for the cat: IC50 COX-1 28.9 μM; IC50 COX-2 0.058 μM; and IC50 COX-1:IC50 COX-2 inhibition ratio 502:1. Thus, there are apparent differences arising from assay methods for both potencies and potency ratios but all available data confirm the selectivity of robenacoxib for COX-2 in the cat and dog.

The pharmacokinetic data in this study demonstrate that robenacoxib was cleared from blood relatively rapidly after both p.o. and subcutaneous dosing, as indicated by MRTs and terminal half-lives of less than 2 h. However, with both administration routes maximum concentrations were achieved rapidly. Relative bioavailability was approximately twice as great after subcutaneous dosing and inter-animal variability was less after subcutaneous administration.

The ex vivo inhibition of COX isoforms followed a time course predictable from the blood concentration-time profiles and a magnitude anticipated from the in vitro studies. Thus, neither robenacoxib by injection nor tablet produced inhibition of COX-1 that is likely to be biologically significant even at early time points with maximal blood concentrations, whereas the control drug meloxicam produced statistically significant inhibition initially and through to the last sampling time at 24 h. On the other hand, both drugs significantly inhibited COX-2 and meloxicam was again active up to 24 h. Consistent with its blood concentration-time profile, robenacoxib inhibited COX-2 more transiently, up to 2 h after p.o. and up to 6 h after parenteral administration. Therefore meloxicam, on these in vitro and ex vivo data can be described as a nonselective COX inhibitor, producing significant inhibition of both isoforms up to and including 24 h at the clinically recommended dose, whereas robenacoxib was clearly COX-2 selective but with a short duration in the central compartment. However, robenacoxib belongs to the class of acidic and highly protein bound NSAIDs which accumulate preferentially at and are slowly cleared from inflamed sites (Lees et al., 2004; Brune & Furst, 2007) and therefore its pharmacodynamic duration of action is longer than predictable from its short residence time in the blood (King et al., 2009). Further studies are required to evaluate the effect of robenacoxib on COX-1 and COX-2 in inflammatory exudates in cats.

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REFERENCES


