In vitro and ex vivo inhibition of canine cyclooxygenase isoforms by robenacoxib: A comparative study

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ABSTRACT

In vitro whole blood canine assays were used to quantify the inhibitory actions of the novel non-steroidal anti-inflammatory drug (NSAID) robenacoxib on the cyclooxygenase (COX) isoenzymes, COX-1 and COX-2, in comparison with other drugs of the NSAID class. COX-1 activity was determined by measuring serum thromboxane (Tx)B2 synthesis in blood samples allowed to clot at 37 °C for 1 h. COX-2 activity was determined by measuring prostaglandin (PG)E2 synthesis in blood samples incubated at 37 °C for 24 h in the presence of lipopolysaccharide. The rank order of selectivity for inhibition of COX-2 versus COX-1 (IC50 COX-1:IC50 COX-2) for veterinary drugs was highest with robenacoxib (128.8) compared to deracoxib (48.5), nimesulide (29.2), S+ carprofen (17.6), meloxicam (7.3), etodolac (6.6), R– carprofen (5.8) and ketoprofen (0.88). Selectivity expressed as the clinically relevant ratio IC50 COX-1:IC50 COX-2 was highest for robenacoxib (19.8) compared to deracoxib (2.3), S+ carprofen (2.5), R– carprofen (2.1), nimesulide (1.8), etodolac (0.76), meloxicam (0.46) and ketoprofen (0.21).

An in vivo pharmacokinetic ex vivo pharmacodynamic study in the dog established dosage and concentration–effect relationships for single oral doses of robenacoxib over the dosage range 0.5–8.0 mg/kg. Values of Cmax and AUC were linearly related to dosage over the tested range. Robenacoxib did not inhibit serum TxB2 synthesis (COX-1) ex vivo at dosages of 0.5–4.0 mg/kg and produced only transient inhibition (at the 1 h and 2 h sampling times) at the 8 mg/kg dosage. All dosages of robenacoxib (0.5–8 mg/kg) produced marked, significant and dose related inhibition of PGE2 synthesis (COX-2) ex vivo.

The data demonstrate that in the dog robenacoxib is a highly selective inhibitor of the COX-2 isoform of COX, and significantly inhibits COX-2 and spares COX-1 in vivo when administered orally over the dosage range 0.5–4.0 mg/kg.

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1. Introduction

The discovery, independently by Vane (1971) and Smith and Willis (1971), that the principal mode of action of non-steroidal anti-inflammatory drugs (NSAIDs) is inhibition of cyclooxygenase (COX), an enzyme in the arachidonic acid cascade, was followed some 20 years later by the identification of a second isoform (Kujubu et al., 1991; Xie et al., 1991). It was initially assumed that most if not all of the well known side-effects of NSAIDs (gastrointestinal ulceration and perforation, renal toxicity, and inhibition of blood clotting and haemostatic pathways) were attributable to inhibition of COX-1, as this isoform is present constitutively in most body cells, erythrocytes being an exception (Masferrer et al., 1994). On the other hand, the newly discovered isoform, COX-2, was found to be absent or present in very low concentra-
ion animal medicine; they include deracoxib and firocoxib (Giere
et al., 1999; McCann et al., 2004; Sessions et al., 2005).

However, pre-clinical studies and clinical experience have indi-
cated that the COXIBs are not free of all side-effects. This is proba-
ably due to three principal factors. First, it is now known that COX-2,
whilst less widespread in its distribution than COX-1, is present
constitutively in some tissues, including the gastric mucosa, kidney
and central nervous system, where it exerts physiological and/or
pathophysiological roles that are the subject of ongoing research
(Wooten et al., 2008). Second, COX-2 is likely to play an important
role in the beneficial process of gastric repair, probably as a result
of the synthesis and release of prostanooids which facilitate repair
processes (Wallace and Devchand, 2005; Wooten et al., 2008).
Third, concentration–inhibition relationships for both COX-1 and
COX-2 have been found to be relatively shallow for many NSAIDs
(Landoni and Lees, 1995; Kay-Mugford et al., 2000; Lees et al.,
2004a; Giraudel et al., 2005; Warner et al., 1999), so that even high
COX-1:COX-2 inhibition ratios of 100:1 or greater do not guarantee
total freedom from inhibition of COX-1 at clinically recommended
dosages (Giraudel et al., 2005). The plasma concentrations pro-
vided by clinically recommended dosages of most NSAIDs are
likely to achieve 80–100% inhibition of COX-2 and, even for selec-
dive drugs, may produce some COX-1 inhibition.

Robenacoxib is a highly selective COX-2 inhibitor, developed for
use in dogs and cats for the suppression of pain and inflammation
(Giraudel et al., 2009a,b; Jung et al., 2009; King et al., 2009). The
aim of this study was to establish the potency of robenacoxib for
COX-1 and COX-2 inhibition in the dog in comparison with other
NSAIDs, including agents currently used in companion animal
medicine. It is well recognised that the degree of NSAID inhibition
of COX isoforms varies with experimental conditions (Lees et al.,
2004a; McCann et al., 2004; Warner et al., 1999). Therefore, in this
investigation, experiments were conducted under standardised
conditions both in vitro and ex vivo.

2. Materials and methods

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

2.1. Comparative in vitro pharmacodynamic study

2.1.1. Animals used and assay methods

This study was undertaken: (1) to assess the inhibitory potency of
robenacoxib against COX-1 and COX-2 in in vitro whole blood as-
says in the dog; and (2) to compare this activity with that of other
reference NSAIDs. For comparison, the following control drugs
were used: R- carprofen, S+ carprofen, celecoxib, deracoxib, dic-
lfenac, etodolac, ketoprofen, meloxicam and nimesulide. Diclofe-
nac was included as it is structurally related to robenacoxib, and
is a COX-2 preferential inhibitor used extensively in human medi-
cine (King et al., 2009). Celecoxib was included, as an example of a
COXIB used in human medicine. All other drugs are licensed for use
in dogs. Both carprofen enantiomers (R- and S+) were studied, be-
cause previous work has shown that they differ markedly in po-
tency as COX-1 and COX-2 inhibitors (Lees et al., 2004a). Each
compound was tested in 5–10 dogs (between 7 and 10 dogs for
most compounds). The primary end-points were the potencies, ex-
pressed as IC50, of the test compounds for inhibition of COX-1 and
COX-2 and the derived COX-1:COX-2 inhibition potency ratios.

From each of 10 beagle dogs (male and female) a volume of 60 mL
blood was taken to enable both assays to be carried out for each concentration of each test compound. All dogs were in
good health, and this was confirmed by haematology and clinical
chemistry analyses. No dog had received any drug for at least
4 weeks prior to study commencement.

For the COX-1 assay, 30 mL blood was collected into S-mono-
vette tubes containing no anticoagulant (Sarstedt AG, Sevelon,
Switzerland). Aliquots of 500 µL were immediately transferred to
tubes containing either 2 µL DMSO (vehicle) or test article dis-
solved in 2 µL DMSO. After mixing, samples were incubated at
37 °C for 60 min. Samples were then centrifuged at 4 °C and
12,000 g for 5 min. The supernatant serum was stored at −20 °C
for a maximum of 1 week prior to analysis of TxB2.

For the COX-2 assay, 30 mL blood was collected into S-mono-
vette tubes containing citrate as anticoagulant (Sarstedt). Aliquots
of 500 µL were immediately pipetted into tubes containing 2 µL
DMSO (vehicle) or test article dissolved in DMSO. After mixing,
a volume of 10 µL lipopolysaccharide (LPS) (at a concentration of
100 µg/mL in phosphate buffered saline) was added to each tube
(LPS 1-2630 from Escherichia coli serotype 0111:B4 was obtained
from Sigma–Aldrich Chemie GmbH, Buchs, Switzerland). Samples
were incubated at 37 °C for 24 h and then centrifuged at 4 °C and
12,000 g for 5 min. The supernatant plasma was stored at −20 °C
for a maximum of 1 week prior to analysis of PGE2.

TxB2 and PGE2 were quantified by enzyme immunoassays using
commercial kits, RPN220-TxB2 Biotrak EIA kit and RPN222-PGE2
Biotrak EIA kit, respectively, obtained from Amersham Pharmacia
(GE Healthcare GmbH, Otelfingen, Switzerland). For both assays
samples were diluted prior to assay.

2.1.2. Data analysis

Plots of serum TxB2 and plasma PGE2 concentration, and the de-
gree of inhibition compared to the control group, versus drug con-
centration were prepared for each test compound. The data were
fitted to Hill plots and the slope (β) and IC50 values calculated.
The extent of parallelism of the curves was assessed. The calcula-
tion of IC50 was based on the standard sigmoidal model:

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

where x = concentration of test substance and y = response, with
four parameters: α, an intercept parameter; β, the slope of the curve
(>0); y0, the y value for x = 0; y1, the y value for x = ∞.

IC50 was defined as the x value for which $y = (y_0 + y_1)/2$, which
yields the equation:

$$IC50 = \exp(-\alpha/\beta)$$

As this model is non-linear, the estimation of its parameters in-
volves an iterative technique, implemented in SAS Proc Nlin
(SAS Online Doc Version 8.2, 1999; SAS Institute Inc., Cary, NC,
USA). Estimates of model uncertainty were then based on linear
approximations.

From the data it was clear that the assumption that $y_1 = 0$ was
plausible; $y_1$ was therefore fixed at zero. $y_0$ varied considerably
between dogs. For data fitting, two models were used as follows:

**Model 1** was fitted to the data for one response, one compound
and one dog. The model fitting procedure was successful in all
cases, but for some the slope was not significantly different from
zero, indicating a poor fit. In addition, the quotient $IC50(TxB_2)/IC50(PGE_2)$ was calculated as a measure of COX-2 selectivity.

Geometric means were determined for all dogs, as well as those
dogs “with good fit”, defined as: (a) slope (β) was significantly dif-
ferent from zero; (b) the estimated value for IC50 did not exceed 10
times the median value for all dogs, with slope significantly differ-
ent from zero and was not less than 1/10 of the median. The med-
ian was determined on the log-scale and backtransformed to the
original linear scale.

**Model 2** was fitted to the data for one response, one compound
and all dogs, with parameters α and $y_0$ varying from dog to dog, but
β being equal for all dogs (common slope model). The model fitting procedure was again successful in all cases, but in one instance the slope was not significantly different from zero, indicating a poor fit. Models 1 and 2 produced qualitatively similar results. Although Model 1 is theoretically superior, with Model 2 more dogs could be taken into account in the analysis. Data are presented for both models, using good fit data only.

Geometric means were computed for all dogs used in the model fitting procedure. In addition, geometric means of the following ratios were calculated as indices of COX-2 selectivity: IC50(TxB2)/IC50(PGE2); IC50/IC10(TxB2); IC50/IC10(PGE2); IC10/IC50(TxB2); IC10/IC50(PGE2).

Slopes of the models for TxB2 and PGE2 inhibition (β) were compared statistically. Using the “average” model for all animals, inhibition of TxB2 was plotted versus inhibition of PGE2.

All calculations were conducted using the software SAS® Version 8.2. In all statistical tests, a two-tailed α level of 0.05 was used.

2.2. In vivo pharmacokinetic and ex vivo pharmacodynamic study

2.2.1. Animals used and assay methods

Twelve beagle dogs (six male, six female) were used in a seven-period cross-over study, conducted in compliance with Good Clinical Practice Guidelines issued by the Committee for Veterinary Medicinal Products [II/3767/92 Final]. All dogs included in the study were in good health, as assessed by clinical examinations and by haematology and blood chemistry analyses. A dose titration study was undertaken to establish, in relation to administered dose in the presence of an acute joint inflammation induced by injection of urate crystals into a stifle joint, the in vivo pharmacokinetics and ex vivo pharmacodynamics of robenacoxib, administered orally in a tablet formulation. Treatments comprised oral administration of placebo tablets, five dosages of robenacoxib (0.5, 1.0, 2.0, 4.0 and 8.0 mg/kg) (administered as 2.5 or 10 mg tablets) and, as a positive placebo tablets, five dosages of robenacoxib (0.5, 1.0, 2.0, 4.0 and 8.0 mg/kg) were compared at each time point using the non-parametric Mann–Whitney U test (Proc npar iway in SAS) since the data were probably not normally distributed.

For the pharmacodynamic data, the seven treatment groups were compared at each time point using the Tukey multiple comparison method. To further evaluate linearity for the variables log AUC(0–6), log AUC(0–∞) and log Cmax, a linear regression to log dosage was performed. For the assumption of linearity to be confirmed, the value of the regression slope β should equal 1, because the dose linearity equation, AUC = α × dosage translates to log AUC = log α + β × log dosage with β = 1.

For the pharmacodynamic data, the seven treatment groups were compared at each time point using the non-parametric Mann–Whitney U test (Proc npar iway in SAS) since the data were probably not normally distributed.

Correlation of TxB2 and PGE2 concentrations with blood concentrations of robenacoxib, using data from animals receiving robenacoxib or placebo, was based on the equation:

\[ \frac{y - y_0}{y_\infty - y} = \alpha + \beta \log \text{conc.} \]

where y is the response (TxB2 or PGE2 concentration relative to pre-value), conc. is the robenacoxib concentration, y0 and y∞ are the response values for concentrations 0 and infinity, respectively; α is an intercept and β > 0 is the slope or Hill coefficient. α, β and y0 were estimated by the model and y∞ was fixed at zero. IC50 was then computed from α and β using the equation:

\[ \log \text{IC}_{50} = -\frac{\alpha}{\beta} \]

with confidence intervals following from Fieller’s Theorem.

3. Results

3.1. Comparative in vitro pharmacodynamic study

Geometric mean IC50 values for COX-1 and COX-2 for the 10 compounds investigated in the whole blood assay using Model 1 are presented in Table 1. For some dogs the slope of the concentration–response relationship could not be differentiated from zero, leading to a poor fit; data are presented only from dogs achieving a good fit. The Model 2 analysis provided a better fit in most cases and data are presented in Table 2, again for data achieving a good fit only. No biologically relevant differences were obtained with results from Models 1 and 2, and from all dogs compared with good fit only. Plots for individual dogs of percentage TxB2 inhibition versus percentage PGE2 inhibition are presented in Fig. 1; the data illustrate relative potencies of COX-1 and COX-2 inhibition and also inter-animal variability for each drug.

Plots of TxB2 and PGE2 concentrations versus blood concentrations of robenacoxib are illustrated in Figs 2 and 3, respectively.

For the eight drugs registered for veterinary use, the rank order of potency for COX-1 (serum TxB2) inhibition was ketoprofen > meloxicam > nimesulide > etodolac > deracoxib > robenacoxib > S+ carprofen > R+ carprofen. For COX-2 (plasma PGE2)
Table 1

<table>
<thead>
<tr>
<th>Drug</th>
<th>PGE₂</th>
<th>TxB₂</th>
<th>Quotient TxB₂/PGE₂</th>
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<tr>
<td></td>
<td>n</td>
<td>Mean</td>
<td>n</td>
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<tr>
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<tr>
<td>Ketoroloxin</td>
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<td>7</td>
</tr>
<tr>
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<td>8</td>
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<tr>
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<td>5</td>
</tr>
<tr>
<td>Robenacoxib</td>
<td>8</td>
<td>0.0429</td>
<td>7</td>
</tr>
</tbody>
</table>

n = number of dogs.

Table 2

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<tr>
<th>Drug</th>
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<th>TxB₂</th>
<th>Quotient TxB₂/PGE₂</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Mean</td>
<td>n</td>
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<tr>
<td>Robenacoxib</td>
<td>9</td>
<td>0.1763</td>
<td>9</td>
</tr>
</tbody>
</table>

n = number of dogs.

inhibition, the corresponding rank potency order was robenacoxib > ketoprofen > meloxicam > deracoxib > nimesulide > etodolac > S+ carprofen > R- carprofen.

The slopes of COX-1 and COX-2 inhibition curves were relatively shallow for all compounds investigated, ranging from 0.938 (nimesulide, COX-1) to 2.73 (R- carprofen, COX-1) and from 1.06 (nimesulide, COX-2) to 2.62 (R- carprofen, COX-2). For robenacoxib slopes were 1.45 (COX-1) and 1.37 (COX-2). For 9 of the 10 compounds tested, curve slopes for inhibition of COX-1 and COX-2 did not differ significantly. The exception was diclofenac, for which the slope was steeper for PGE₂ inhibition (p = 0.0083).

Differences in slopes of the inhibition curves for TxB₂ and PGE₂, even when not statistically significant, led to small differences in estimates of potency ratios, depending on the level of inhibition considered (Table 3). Of the eight compounds registered for veterinary use, the rank order of potency for inhibition ratios, I₅₀ COX-1:IC₅₀ COX-2, reflecting selectivity for COX-2, was robenacoxib > deracoxib > nimesulide > S+ carprofen > meloxicam > etodolac > R- carprofen > ketoprofen. The same rank order was obtained for IC₅₀ inhibition ratios except for interposition between etodolac and meloxicam. The IC₅₀ TxB₂/IC₅₀ PGE₂ ratio provides a particularly useful indicator of selectivity, since in clinical use it is generally regarded as appropriate to achieve IC₅₀ concentrations for COX-2 inhibition to ensure clinical efficacy, whilst it is considered desirable for concentrations not to exceed the IC₅₀ for COX-1 inhibition to ensure minimal side-effects in relation to the gastrointestinal tract and haemostasis (Giraudel et al., 2005, 2009a). Of the drugs of veterinary interest, the highest ratio (19.8) was obtained for robenacoxib and the lowest (0.21) was obtained for ketoprofen (Table 3).

3.2. In vivo pharmacokinetic and ex vivo pharmacodynamic study

3.2.1. Pharmacokinetics

Blood pharmacokinetic variables for each dosage group of robenacoxib are presented in Table 4. For AUC[0–6], AUC[0–∞] and Cₘₐₓ, normalised for administered dosage, mean, median and range values were similar for all five robenacoxib dosages, except for a small numerical increase in AUC values at the highest dosage (8 mg/kg), but which did not differ significantly from values obtained with the four lower dosages (vide infra). For all dosages of robenacoxib there were no significant differences between female and male dogs.

Median and range for Tₘₐₓ were 1 h and 0.5–4.0 h, respectively. The terminal half-life in blood was relatively short (harmonic mean values in the range 0.60–0.91 h) and non-dose related.

The linear model used to assess differences based on treatment, sex and treatment × sex for log-transformed data depends on the assumption of normality. Therefore, the standardised residuals were subjected to a test for normality. Excluding an outlier value for one treatment in a single dog, all variables except Tₘₐₓ were normally distributed as follows: log AUC[0–6], p = 0.97; log AUC[0–∞], p = 0.64; log Cₘₐₓ, p = 0.86; log Tₘₐₓ, p = 0.42; log Tₜₐₐₜ, p = 0.28. The latter value is not unexpected, as values are discrete (0.5, 1, 2 or 6 h). None of the model parameters (treatment, sex and treatment × sex) differed significantly for any pharmacokinetic variable. The analyses also indicated no linear or non-linear dependency on log dosage.

Based on the Tukey multi-comparison test for all possible comparisons, no group comparisons yielded a significant difference, although p = 0.055 for one comparison (robenacoxib 1 mg/kg versus 4 mg/kg for Tₜₐₐₜ). Therefore, none of the variables AUC[0–6]/dosage, AUC[0–∞]/dosage, Cₘₐₓ/dosage, Tₘₐₓ and Tᵢₜ differed significantly between either dosages of robenacoxib or sexes. Linearity was confirmed using linear regression of log AUC[0–6], log AUC[0–∞] and log Cₘₐₓ versus log dosage. Slope estimates were 1.027 (log AUC[0–6]), 1.028 (log AUC[0–∞]) and 1.002 (log Cₘₐₓ) and 95% confidence intervals were narrow (data not shown), confirming the assumption of normality for these variables.

3.2.2. Pharmacodynamics

The magnitude and time course of ex vivo inhibition of synthesis of TxB₂ and PGE₂ are presented in Table 5. The significance of each of the differences at times of 1, 2 and 6 h between each of the five dosages of robenacoxib and placebo and meloxicam treatments is indicated in Table 6. The pre-dosing values of TxB₂ and PGE₂ were not significantly different between each of the seven groups (p > 0.54 for serum TxB₂, p > 0.16 for plasma PGE₂). Analysis of observed values is therefore more relevant in this case than analysis of changes from baseline, as the latter introduces an additional source of error. Observed values were therefore used as the primary parameter. The data for placebo dosing indicate relative constancy of TxB₂ concentration between 0 and 6 h but there was an increase with time in PGE₂ concentration at 2 and 6 h. Therefore, for statistical analyses the data for meloxicam and robenacoxib groups were evaluated relative to the placebo.

The positive control drug, meloxicam, produced no significant change compared to placebo in TxB₂ concentration at any sampling time (Tables 5 and 6). Compared to meloxicam but not placebo, the highest dosage of robenacoxib produced slight but significant inhibition of serum TxB₂ at 1 h but not at 2 h or 6 h (Tables 5 and 6).

Meloxicam produced no significant change compared to placebo in PGE₂ concentration at any sampling time (Tables 5 and 6). In comparison with both placebo and meloxicam treatments, robenacoxib at every dosage significantly inhibited PGE₂ synthesis at times of 1 and 2 h after dosing, when assessed as absolute ng/mL.
values (Tables 5 and 6). The differences from both placebo and meloxicam were also statistically significant for all robenacoxib dosages at both 1 and 2 h when expressed as percentage change, except for the lowest dosage (0.5 mg/kg) at a single time of 2 h.
At both 1 and 2 h, robenacoxib inhibition of PGE2 was both dosage and blood concentration dependent. Values of maximal PGE2 inhibition compared to baseline were:

- +4.8% (placebo); 
- +10.5% (meloxicam); 
- +50.0% (0.5 mg/kg robenacoxib); 
- +65.1% (1.0 mg/kg robenacoxib); 
- +79.1% (2.0 mg/kg robenacoxib); 
- +84.5% (4.0 mg/kg robenacoxib); 
- +84.2% (8.0 mg/kg robenacoxib).

The action of robenacoxib on PGE2 synthesis was relatively short-lived, as indicated by no significant differences from both placebo and meloxicam at 6 h, except for significant inhibition relative to meloxicam at this time for the 4 mg/kg robenacoxib dosage ($p = 0.036$, Table 6).

For TxB2, maximal inhibition was not obtained in any dog and a good fit of the data to blood robenacoxib concentration could not be established. However, a reliable fit for blood robenacoxib concentration and PGE2 inhibition was achieved. The slope ($\beta$) was 1.3, and IC50 was 280 ng/mL.
ED20 ratio COX-1:COX-2 for robenacoxib was 94; ED20 was selected as a more accurate measure than the ED50 ratio for this data set.

For COX-2 inhibition (PGE2) estimated oral dosages of robenacoxib were: ED20 = 0.048 mg/kg; ED50 = 0.34 mg/kg; ED90 = 2.38 mg/kg. Because of limited inhibition of COX-1 (TxB2) by robenacoxib, even with the highest dosage of 8 mg/kg, less precise values were determined as follows: ED20 = 4.52 mg/kg; ED50 = 17.4 mg/kg. The ED30 ratio COX-1:COX-2 for robenacoxib was 94; ED30 was selected as a more accurate measure than the ED50 ratio for this data set.

4. Discussion

4.1. Comparative in vitro pharmacodynamic study

Quantitative determination of concentration–effect relationships for COX-1 and COX-2 inhibition by NSAIDs has several uses (Lees et al., 2004a,b). First, it establishes levels of \( t_{50\%} \) against each COX isof orm, and thus indicates if 100% inhibition is attainable. Secondly, it establishes levels of potency, usually defined as IC50 but, in the case of NSAIDs, more appropriately defined as a value for COX-1 inhibition of 20% or less (as an indirect index of safety) and a higher value for COX-2 of 80–95% (as an indirect predictor of efficacy). Thirdly, based on relative potencies of COX-1 and COX-2 inhibition, quantitative data allow determination of several COX-1:COX-2 potency ratios e.g. IC50 COX-1:IC50 COX-2, IC50 COX-1:IC50 COX-2; IC20 COX-1:IC20 COX-2, as shown by Giraudel et al. (2005 and 2009a). Whilst the numerical values of potency ratios do not precisely correlate with the margin of safety of NSAIDs in clinical use, it is widely accepted that a high level of selectivity for COX-2 is likely to be associated with lack of interference with haemostasis and with limited likelihood of gastrointestinal (and possibly renal) side-effects (Warner et al., 1999). Fourthly, establishing the whole sweep of the concentration–effect relationships allows calculation of the third pharmacodynamic parameter, the slope.

There are considerable differences in the scientific literature of values for individual NSAIDs, in respect of both potency (usually expressed as IC50 values for COX-1 and COX-2) and COX selectivity (expressed as IC50 COX-1:IC50 COX-2 ratios). As discussed by Warner et al. (1999) and Lees et al. (2004a,b), this variability within and between laboratories is in part attributable to experimental conditions, and are therefore the most useful for predicting the clinical relevance of given levels of COX-1 and COX-2 inhibition (Patrignani et al., 1994; Brideau et al., 2001; Warner et al., 1999) and the dog (16.8, Streppa et al., 2002; 8.0, McCann et al., 2004; 5.4, Wilson et al., 2004; 6.5, Brideau et al., 2004). Interpreting data for the racemate of carprofen is, however, of limited value, because it is a mixture of two drugs, each with differing potencies for COX-1 and COX-2 inhibition and hence differing COX-1:COX-2 ratios. Using the more active S+ enantiomer of carprofen, Lees et al. (2004b) obtained an IC50 COX-1:COX-2 ratio of 25 in a canine whole blood assay, which agrees well with the value of 18 obtained in this study and with the value of 16.8 reported by Streppa et al.
In all cases of significance (indicated by bold type), values comparing robenacoxib with placebo and meloxicam, and meloxicam versus placebo, were determined by the Mann–Whitney U test. Significant differences between baseline at each time point in dogs receiving single oral doses of placebo, robenacoxib or meloxicam.

Table 5
Univariate statistics for serum TxB2 and PGE2 concentrations in whole blood assays (as indices of COX-1 and COX-2 activity respectively) and percentage inhibition relative to baseline at each time point for TxB2 and PGE2 concentrations and percentage inhibition in whole blood assays (as indices of COX-1 and COX-2 activity respectively) in dogs receiving single oral doses of placebo, robenacoxib or meloxicam.

<table>
<thead>
<tr>
<th>Variable (units)</th>
<th>Time (h)</th>
<th>Placebo</th>
<th>Robenacoxib</th>
<th>Meloxicam</th>
</tr>
</thead>
<tbody>
<tr>
<td>TxB2 (ng/mL)</td>
<td>0</td>
<td>0.95</td>
<td>1.00</td>
<td>0.70</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>0.05</td>
<td>1.00</td>
<td>0.70</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.25</td>
<td>1.00</td>
<td>0.70</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>0.06</td>
<td>1.00</td>
<td>0.70</td>
</tr>
<tr>
<td>TxB2 inhibition</td>
<td>0</td>
<td>0.09</td>
<td>0.0079</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>0.00</td>
<td>0.001</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.02</td>
<td>0.001</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>0.06</td>
<td>0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>PGE2 (ng/mL)</td>
<td>0</td>
<td>0.48</td>
<td>0.10</td>
<td>0.17</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>0.05</td>
<td>0.17</td>
<td>0.17</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.02</td>
<td>0.001</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>0.06</td>
<td>0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>PGE2 inhibition</td>
<td>0</td>
<td>0.012</td>
<td>0.001</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>0.013</td>
<td>0.001</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.013</td>
<td>0.001</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>0.06</td>
<td>0.001</td>
<td>0.001</td>
</tr>
</tbody>
</table>

The usefulness of the whole blood assay used in this investigation is demonstrated by the selective inhibition of COX-2 (>20-fold selectivity for IC50 ratios) shown for robenacoxib, deracoxib, nimesulide and celecoxib, the preferential inhibition of COX-2 (>1–20-fold selectivity) shown for R− and S+ carprofen, diclofenac, etodolac and meloxicam, and non-selectivity (<1-fold selectivity) of ketoprofen. Moreover, from a perspective of predicting efficacy and especially safety in clinical use, the ratio IC50 COX-1:IC50 COX-2, may be more useful than the IC50 COX-1:IC50 COX-2 ratio. Potency ratios for IC50 COX-1:IC50 COX-2 were markedly higher for robenacoxib (19.8) compared to values for deracoxib (5.3), S+ carprofen (2.5), R− carprofen (2.1), nimesulide (1.8), etodolac (0.76), meloxicam (0.46) and ketoprofen (0.21). In future studies, it will be of interest to compare robenacoxib potency ratios with those of other COXIBs recently introduced into veterinary medicine, such as firocoxib and mavacoxib which were not available to us at the time this study was undertaken. In summary, the present data indicate that carprofen may be classified as a drug of low potency, and ketoprofen and meloxicam as drugs of high potency.
against both COX isoforms, whereas robenacoxib possessed high potency for COX-2 but low potency for COX-1 inhibition.

4.2. Pharmacokinetic–pharmacodynamic study

The three parameters of any drug which characterise it pharmacodynamically are efficacy ($I_{\text{max}}$), potency ($IC_{50}$, $IC_{90}$, $IC_{95}$ etc.) and slope ($\beta$) (Toutain and Lees, 2004; Lees et al., 2004b). Each is crucially important for clinical use, but for differing reasons. Efficacy is important to the clinician because it defines the maximum response attainable. For most NSAIDs efficacy is probably very similar, so that provided dosages are sufficiently high there is likely to be no difference in the degree of efficacy attained. Potency is of less relevance to the clinician, but is crucial to the pharmaceutical company which is charged with setting a dosage level to achieve a desirable degree of efficacy. Toxicity also becomes an issue if dosage is high relative to the safety margin. Slope determines sensitivity and is also potentially important in determining selectivity between desirable therapeutic effects and toxic actions.

In the present investigation the blood $C_{\text{max}}$ for orally administered robenacoxib at a dosage of 2 mg/kg was 1166 ng/mL (3.56 µM). A $C_{\text{max}}$ value of 947 ng/mL (2.89 µM) was reported by Jung et al. (2009) in normal dogs administered robenacoxib at a lower dosage of 1 mg/kg in the fasted state. Comparing these values to the $IC_{50}$ for COX-2 inhibition determined using Model 2 (0.079 µM), it may be noted that the $C_{\text{max}}$ values are 45 and 37 times greater, respectively. Relative to the $IC_{50}$ for COX-2 inhibition based on Model 1 (0.043 µM), they are 83 and 67 times greater, respectively. In contrast, the corresponding multiples of $IC_{50}$ for COX-1 inhibition (10.77 µM) determined by method 2 were 0.33 and 0.27, respectively. Therefore, these in vitro data confirm that the peak blood concentrations of robenacoxib achieved in vivo after oral dosing with clinically recommended dosages (1–2 mg/kg) provide marked inhibition of COX-2 and minimal inhibition of COX-1, so that the high selectivity demonstrated in vitro will also occur in vivo.

Potency values may be used, in conjunction with plasma or blood concentration–time data, to determine the magnitude and time course of COX-1 and COX-2 inhibition provided by given dose schedules of given products administered by a given route (Giraudel et al., 2009a,b; King et al., 2009). Drug potency may also be used, together with clearance and bioavailability, to establish a dosage required to produce an average blood concentration providing a given level of COX-1 or COX-2 inhibition, based on the equation.

\[
\text{Dose} = \frac{\text{Cl} \times IC_{x}}{F}
\]

where $x$ can have any value from 0% to 100% (Toutain and Bousquet-Melou, 2004; Toutain and Lees, 2004).

Using the pharmacokinetic data derived by Jung et al. (2009) for robenacoxib in the dog (clearance after intravenous dosing = 0.81 L/kg/h, bioavailability after oral dosing = 0.84), the dosage of robenacoxib providing an average blood concentration for 24 h after a single dose equal to the in vitro determined $IC_{50}$ value (Model 2, 0.079 µM) is 0.60 mg/kg. Use of the $IC_{50}$ to predict the clinical dosage (Lees et al., 2004a; Warner et al., 1999) leads to a suggested daily dosage of 1.6 mg/kg, which is close to the middle of the oral dosage range of 1–2 mg/kg, which was independently confirmed to be effective and well tolerated in clinical trials in dogs (Novartis data on file).

Meloxicam, the positive control drug used in the ex vivo study, produced slight but non-significant inhibition of TxB2 and exerted no effect on PGE2 synthesis. The lack of effect on TxB2 is not surprising, as meloxicam is generally regarded as a preferential inhibitor of COX-2 (Giraudel et al., 2005; Warner et al., 1999). On the other hand, the failure of meloxicam to inhibit PGE2 was an unexpected finding for the same reason and also in light of the findings of previous workers (Brideau et al., 2001). The lack of effect of meloxicam in this study is not attributable to any deficiency in the model, its validity being clearly confirmed by the significant maximum inhibitory actions of robenacoxib on PGE2 of 79.1%, 84.5% and 84.2%, for dosages of 2, 4 and 8 mg/kg, respectively, indicating that the 2 mg/kg dosage achieved near maximal attainable inhibition.

The ex vivo data indicate a relatively short duration of action of robenacoxib in the central compartment, as inhibition of PGE2 was weak or absent at the 6 h sampling time. However, the duration of clinical efficacy of robenacoxib is predicted to be significantly longer, as robenacoxib concentrates in and has a long residence time in inflammatory exudate (King et al., 2009).

The present study in fasted, healthy male and female beagle dogs demonstrated that orally administered robenacoxib was rapidly absorbed and eliminated; the median $T_{\text{max}}$ was 1 h and the harmonic mean for terminal blood half-life ranged from 0.60 to 0.91 h. As a relatively sparse sampling protocol was used, derived pharmacokinetic variables should not be regarded as precise, but the sampling times were nevertheless sufficient to provide acceptable approximations for all reported variables. Over the dosage range 0.5–8.0 mg/kg, $C_{\text{max}}$, AUIC[0–6] and AUIC[0–∞] were not significantly different when normalised for dosage. Therefore, these pharmacokinetic variables increased in direct proportion to administered dosage of robenacoxib.

References


