Differential pharmacokinetics and pharmacokinetic/pharmacodynamic modelling of robenacoxib and ketoprofen in a feline model of inflammation

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Robenacoxib and ketoprofen are acidic nonsteroidal anti-inflammatory drugs (NSAIDs). Both are licensed for once daily administration in the cat, despite having short blood half-lives. This study reports the pharmacokinetic/pharmacodynamic (PK/PD) modelling of each drug in a feline model of inflammation. Eight cats were enrolled in a randomized, controlled, three-period cross-over study. In each period, sterile inflammation was induced by the injection of carrageenan into a subcutaneously implanted tissue cage, immediately before the subcutaneous injection of robenacoxib (2 mg/kg), ketoprofen (2 mg/kg) or placebo. Blood samples were taken for the determination of drug and serum thromboxane (Tx)B₂ concentrations (measuring COX-1 activity). Tissue cage exudate samples were obtained for drug and prostaglandin (PG) E₂ concentrations (measuring COX-2 activity). Individual animal pharmacokinetic and pharmacodynamic parameters for COX-1 and COX-2 inhibition were generated by PK/PD modelling. S(+) ketoprofen clearance scaled by bioavailability (CL/F) was 0.114 L/kg/h (elimination half-life = 1.62 h). For robenacoxib, blood CL/F was 0.684 L/kg/h (elimination half-life = 1.13 h). Exudate elimination half-lives were 25.9 and 41.5 h for S(+) ketoprofen and robenacoxib, respectively. Both drugs reduced exudate PGE₂ concentration significantly between 6 and 36 h. Ketoprofen significantly suppressed (>97%) serum TxB₂ between 4 min and 24 h, whereas suppression was mild and transient with robenacoxib. In vivo IC₅₀/COX-1/IC₅₀/COX-2 ratios were 66.9:1 for robenacoxib and 1:107 for S(+) ketoprofen. The carboxylic acid nature of both drugs may contribute to the prolonged COX-2 inhibition in exudate, despite short half-lives in blood.

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INTRODUCTION

Nonsteroidal anti-inflammatory drugs (NSAIDs) inhibit cyclooxygenase (COX) and have been used for many decades to alleviate inflammation-related pain in human and veterinary medicine. COXibs belong to a class of NSAIDs that selectively inhibit the COX isomform up-regulated in inflammation (COX-2), with much less inhibition of the constitutively expressed COX isomform (COX-1) responsible for the production of so-called ‘housekeeping’ eicosanoids. Several COXibs were associated with an increased risk of myocardial infarction or stroke in man when evaluated against nonselective NSAID comparators and administered at recommended dose rates (Bombardier et al., 2000; Silverstein et al., 2000). However, lumiracoxib contrasted with other COXibs by displaying favourable cardiovascular and gastrointestinal safety profiles in a study incorporating more than 18 000 human patients (Farkouh et al., 2004).

Despite a short plasma half-life (2–6.5 h) in man, lumiracoxib was authorized for once daily administration (Mysler, 2004), whereas rofecoxib and celecoxib were both administered twice daily, despite their long respective half-lives of 17 and 19–32 h (Vasquez-Bahena et al., 2010). The persistent clinical efficacy of lumiracoxib may be related to a prolonged...
Robenacoxib is a structural analogue of lumiracoxib, licensed for use in cats (and dogs). Both drugs are structurally related to diclofenac (Fig. 1), containing a carboxylic acidic function instead of the methylsulphone (as for rofecoxib) or the sulphonamide (as for celecoxib) groups characteristic of first generation COXibs. Robenacoxib has a short blood half-life of 1.9 h after subcutaneous administration in the cat (Pelligand et al., 2012b) but is as effective as an analgesic as meloxicam (half-life 37 h) for at least 22-h post-operatively (Kamata et al., 2012).

Ketoprofen is a COX-1 selective NSAID (Fig. 1), licensed for once daily administration in man and cats (Warner et al., 1999; Schmid et al., 2010) despite short elimination half-lives for the eutomer S(+)-ketoprofen of 1.2 h in the cat and 2–3 h in man (Rudy et al., 1998; Lees et al., 2003).

We hypothesized that robenacoxib and ketoprofen would be similarly effective as anti-inflammatory drugs in a feline model of inflammation, because of the differences in COX selectivity, because of similar concentration time profiles and potency for COX-2 inhibition. Several models of inflammation have been developed in the cat (Giraudel et al., 2005b; Pelligand et al., 2012a) but only the carrageenan-based tissue cage model of Pelligand et al. (2012a) allows serial measurement of NSAID concentrations in blood and at the site of inflammation, as well as determination of the magnitude and time-profiles of COX-1 and COX-2 inhibition. We have previously described exudate sampling from these cages for serial measurement of NSAID and Prostaglandin (PG)E2 concentrations, the latter inflammatory mediator as a surrogate of COX-2 activity, together with blood sampling for the measurement of serum thromboxane (Tx)B2 as a surrogate of COX-1 activity (Pelligand et al., 2012a,b). The model further enables calculation of pharmacodynamic parameters of NSAIDs, namely \( I_{\text{max}} \) (efficacy), \( IC_{50} \) (potency) and \( n \) (slope of the concentration-effect relationship). These parameters are used to calculate dosage regimens for clinical use (Lees et al., 2004).

The aims of this study were to: (i) compare the pharmacokinetic and the pharmacodynamic profiles of robenacoxib and ketoprofen in feline blood and exudate; (ii) compare in vivo IC\(_{50}\) COX-2, ex vivo IC\(_{50}\) COX-1 and COX inhibition selectivity for each drug; and (iii) compare and contrast the pharmacokinetic and pharmacodynamic profiles of two carboxylic acid sub-groups of NSAIDs, the profens and COXibs, in the cat.

MATERIALS AND METHODS

Animals

Eight domestic short hair cats (all neutered, five males and three females, aged 1–3 years), weighing 4.0 ± 0.39 kg, were enrolled into the study after an acclimatization period of 1 month. Health checks were performed before the start of each sampling period. Between study periods, the cats were group housed altogether. They were fed a dry commercial diet (RF23, Royal Canin, Aimargues, France) in two equal portions daily, based on their metabolic requirements. Drinking water was available ad libitum. The room was lit from 7.00 am to 7.00 pm.

The study complied with United Kingdom Home Office regulations (Project License Number 70/6132). The protocol was approved by the Royal Veterinary College Ethics and Welfare Committee. All tissue cages were electively removed under general anaesthesia 6 months after implantation, before the cats were rehomed, as no long-lasting sequelae resulted from the protocol.
Animal preparation and induction of inflammation

Four tissue cages were implanted surgically in each cat, as previously described (Pelligand et al., 2012b). Briefly, medical grade silicone cylindrical tissue cages (SF Medical, Fresno, CA, USA) were prepared to the following dimensions: 70 mm length, 15.9 mm external diameter, 12.7 mm internal diameter (6.7 cm³ internal volume) with 12 holes at each pole providing a total surface exchange area of 3.0 cm² per cage. They were sterilized and surgically inserted subcutaneously, under isofurane (IsoFLO, Abbott Animal Health, Maidenhead, UK) general anaesthesia, parallel to the vertebral column in the flank and thorax areas. Analgesia was provided intra- and postoperatively as described by Pelligand et al. (2012a). The tissue cages were flushed with sterile saline under general anaesthesia 7 weeks after implantation to remove any remaining cellular debris and subsequently used experimentally, not earlier than 10 weeks after implantation. On the day before dosing, the fur over the cages was clipped and a double-lumen catheter (CS15402E, Arrow International Ltd, Uxbridge, UK) was inserted in a jugular vein under general anaesthesia (Pelligand et al., 2012a).

On day 1 of each period, 1 mL of a 2% sterile carrageenan solution (Viscarin, FMC biopolymers, Philadelphia, PA, USA) was injected into a naive tissue cage (Pelligand et al., 2012b), and this tissue cage was used to harvest exudate in that period. A different tissue cage was stimulated for each subsequent period.

Experimental design

The experiment was conducted as a three period, three sequence, cross-over design with 28-day washout intervals. The treatment for the first period was allocated following a randomized blocked design and the sequence of treatments for the subsequent periods followed an incomplete latin square design. All cats received each of the treatments. The three treatments, administered subcutaneously in the neck area, were as follows: robenacoxib 2 mg/kg (Onsior 2.0% solution, Novartis Animal Health, Basel, Switzerland); racemic ketoprofen 2 mg/kg (Ketolen 1% solution, Merial Animal Health, Harlow, Essex, UK); and 0.9% saline (0.1 mL/kg) as placebo. On each test day, the cats were fed 2 h before dosing and again after the final blood sample of the day (12 h after dosing). The carrageenan-stimulated samples were sampled (1.0–1.3 mL of exudate on each occasion) before and at 3, 6, 9, 12, 24, 34, 48, 72, 96 and 120 h after carrageenan injection (11 serial samples from the same cage). Samples were transferred immediately to 1.5-mL Eppendorf tubes containing 10 μg indomethacin (Sigma Aldrich, Poole, Dorset, UK) to prevent artefactual ex vivo eicosanoid generation. The tube was mixed by gentle inversion and placed on ice until centrifugation at 1000 g, 4 °C for 10 min. The supernatant was aliquoted and frozen at −80 °C prior to measurement of exudate PGE₂ and concentrations of ketoprofen or robenacoxib.

Blood samples (maximum 1.5 mL per sample) were taken from the distal port jugular catheter before dosing and at 4, 15, 30 min, then 1, 1.5, 2, 3, 4, 6, 9, 12, 24 and 48 h after dosing. An aliquot of each blood sample (0.2 mL) was allowed to clot in a glass tube (Chromacol, Welwyn Garden City, UK) while incubated in a water bath at 37 °C for 1 h then centrifuged (1500 g, 4 °C, 10 min) and the supernatant stored at −80 °C prior to measurement of serum TxB₂. The remainder of the sample (1.3 mL) was transferred into an EDTA tube (International Scientific Supplies Ltd., Bradford, UK) for blood robenacoxib measurement or a heparin tube for plasma ketoprofen measurement and stored at −80 °C.

To ensure accuracy of pharmacokinetic calculations, cats were weighed on the day of catheter placement, actual injected doses were calculated by weighing syringes before and after injection and actual rather than nominal times of blood sampling were used.

Measurement of NSAID concentrations

Plasma (ketoprofen), blood (robenacoxib) and exudate (both drugs) were spiked with known drug concentrations to establish standard curves, and quality controls (QCs) were prepared and dispersed over the sequence of unknown samples, to monitor the overall performance of each analytical method. The percentage of back-calculated concentrations of standards within ±15% of their nominal value and the percentage of QCs within ±15% of their theoretical value were calculated. Imprecision (indicator of between day repeatability) was expressed as the coefficient of variation (CV%) between standard concentrations run on different days. Inaccuracy was expressed as the deviation of the mean (% Relative Error) from the theoretical concentration spiked into blank matrix.

Robenacoxib concentrations in feline blood were measured using a sensitive analytical method, as described by Jung et al. (2009). Briefly, the method involved an initial analysis by HPLC-UV, covering the range of 500–20 000 ng/mL and, if required, a subsequent analysis by LC-MS, covering the range of 3–100 ng/mL for blood. Depending on the results obtained by UV analysis, samples were diluted if necessary in order not to exceed a concentration of 100 ng/mL in the MS method. The same method was used for exudate, except that 250 μL of sample were extracted and diluted twofold with water, instead of using 500 μL of blood. For blood with the MS method, the lower and upper limits of quantification were 3 and 100 ng/mL, respectively. As the exudate was diluted twofold, the MS method had a range of 6–200 ng/mL for exudate in the initial method validation, but this was extended subsequently to 3.5–200 ng/mL, as it was established during the analysis that reliable results were obtained at the lower end of the range. For robenacoxib, inaccuracy was <10.4%, and imprecision was <9.3%.

R(−) ketoprofen and S(+) ketoprofen concentrations were measured in exudate and plasma by liquid chromatography-mass-spectrometry (API 2000 LC/MS/MS system, Applied Biosystems, Streetsville, ON, Canada). The method, previously validated for cat and piglet plasma (Fosse et al., 2011 and Hormazábal, unpublished data), had lower and upper limits of quantification of 10 and 8000 ng/mL for both matrices. After extraction, filtration and centrifugation of 0.5 mL...
pharmacodynamic measurements

In vivo generation of exudate PGE₂ and ex vivo generation of serum TxB₂ were used as surrogates for COX-2 and COX-1 activities, respectively. Although the main source of TxB₂ in serum is platelet COX-1, a minor contribution from COX-2 or from other cells cannot be completely excluded. Additionally, COX-1 may contribute to the synthesis of PGE₂ in exudate (Nantel et al., 1999; Wallace et al., 1999), but the magnitude of this production is likely to be negligible, based on the fact that COX-1 is not induced in carrageenan inflammatory models (Tomlinson et al., 1994).

Serum TxB₂ and exudate PGE₂ concentrations were measured with competitive radio-immunoassays, adapted from Higgins and Lees (1984) as described in a previous validation paper (Pellegand et al., 2012a). Two concentrations of pooled samples were aliquoted and used as quality controls, dispersed over the sequences of unknown samples to calculate inter- and intra-assay variability. Exudate PGE₂ intra-assay variability was 3% for the high control concentration (2.9 ng/mL) and 23.9% for the low control concentration (0.13 ng/mL). Interassay variability was 2.1% for the high control and 31% for the low control concentrations. Serum TxB₂ intra-assay variability was 7.2% for the high control (235.1 ng/mL) and 13.2% for the low control (56.5 ng/mL) concentrations. Interassay variability was 2.3% and 11.3% for the high and low control concentrations, respectively. All validation data complied with analytical recommendations guidelines (Kelley & DeSilva, 2007; Viswanathan et al., 2007) except for PGE₂ interassay variability. Therefore, all samples from the same cats were always analysed in the same batch.

Pharmacokinetic data analysis

Pharmacokinetics and PK/PD modelling were performed by the least-squares regression method, using commercial software (WINNONLIN version 5.2, Pharsight Corporation, Mountain View, CA, USA). Goodness of fit and selection of the appropriate model were evaluated using the Akaike Information Criterion estimate (Yamaoka et al., 1978) and by visual inspection of the fitted curves and residuals.

Blood robenacoxib and plasma ketoprofen enantiomer concentrations C(t) were fitted for each cat using an equation corresponding to drug disposition in a two-compartmental model with absorption phase (subcutaneous administration, equation 1):

\[
C(t) = - (Y_1 + Y_2) e^{-k_1(t-tlag)} + Y_1 e^{-\lambda_1(t-tlag)} + Y_2 e^{-\lambda_2(t-tlag)}
\]

(1)

Where \(\lambda_1\) and \(\lambda_2\) are the initial and terminal slopes (1/h), \(Y_1\) and \(Y_2\) the intercepts on the Y axis (ng/mL) when C(t) is plotted on a semi-logarithmic scale, \(k_1\) is the first-order absorption rate constant (1/h) and tlag the absorption lag time after subcutaneous administration. Data were weighted by the reciprocal of the estimated value for blood or plasma concentration when necessary.

Exudate concentrations of robenacoxib or ketoprofen enantiomers Cₑ(t) were fitted for the data from each cat using an equation corresponding to drug disposition in a bicompartimental model with an absorption phase after dose normalization (equation 2):

\[
Cₑ(t) = - (Yₑ₁ + Yₑ₂) e^{-kₑ(t-tlag)} + Yₑ₁ e^{-\lambdaₑ₁(t-tlag)} + Yₑ₂ e^{-\lambdaₑ₂(t-tlag)}
\]

(2)

Where \(\lambdaₑ₁\) and \(\lambdaₑ₂\) are the initial and terminal slopes (1/h), \(Yₑ₁\) and \(Yₑ₂\) the intercepts on the Y axis (ng/mL) when Cₑ(t) is plotted on a semi-logarithmic scale, and \(kₑ\) is the first-order invasion rate constant in exudate (1/h). No weighting was applied to the data for fitting. It was assumed that only a negligible amount of each NSAID gained access to the tissue cage and that the pharmacokinetics in exudate had no effect on the time course of drug disposition in the rest of the body.

Pharmacokinetic parameters were generated for robenacoxib (in blood and exudate) and S(+)- and R(−)-ketoprofen (in plasma and exudate) by noncompartmental analysis for individual cats as follows: Maximum NSAID concentration, \(C_{max}\), Time of maximum NSAID concentration, \(t_{max}\), Area under NSAID concentration-time curve, \(AUC₀⁻inf\), Area under the Moment Curve, \(AUMC₀⁻inf\), NSAID Mean Residence Time (MRT) = \(AUMC₀⁻inf/AUC₀⁻inf\). NSAID terminal half-life, \(t₁/₂ = \ln(2)/\lambdaₑ\). Where \(\lambdaₑ\) is the slope of the drug elimination phase, computed by linear regression of the logarithmic concentration versus time curve during the elimination phase. NSAID clearance scaled by bioavailability (\(F\)), \(CL/F\) = dose/\((F \times AUC₀⁻inf)\). Where \(F\) is the bioavailability for extravascular administration. Apparent volume of distribution of NSAID during the elimination phase, \(Vₑ_{app}/F\) = (dose/\(F\))/(\(AUC₀⁻inf \times \lambdaₑ\)).

Pharmacodynamic data analysis and PK/PD modelling

A user program was purposely written in WINNONLIN for PK/PD modelling. The equations of robenacoxib and S(+) ketoprofen enantiomer disposition in blood/plasma C(t) or in exudate Cₑ(t) were obtained by compartmental pharmacokinetic analysis by fitting equations (1) or (2), respectively, to the observed data. Individual pharmacokinetic parameters were entered as constants to solve the PK/PD models in a two-stage analysis (Giraudel et al., 2005a).
In vivo generation of exudate PGE₂ was used as a surrogate for COX-2 activity to carry out PK/PD modelling of the NSAIDs in exudate (Lees et al., 2004). An indirect response model described by Pelligand et al. (2012b) was used to model the effect of robenacoxib and S(+) ketoprofen on exudate PGE₂ production. The model did not include the R(−) enantiomer, as it was considered to be devoid of activity on cyclooxygenase at the concentrations achieved. Indeed, S(+) ketoprofen is the enantiomer of the S(+)R(−) ketoprofen enantiomeric pair (Lees et al., 2003). The response is indirect because it is the consequence of a dynamic physiologic equilibrium between PGE₂ production after carrageenan injection, the natural clearance of PGE₂ from exudate and the reversible inhibition of COX-2 by NSAIDs, preventing the build-up of PGE₂ in exudate as in equation 3 (Dayneka et al., 1993):

\[
\frac{d\text{PGE}_2}{dt} = K_{in}(t) - K_{out} \times \text{PGE}_2
\]  

Where \(d\text{PGE}_2/dt\) (ng/mL/h) is the rate of change of PGE₂ concentration in exudate, \(K_{out}\) (1/h) is a first-order parameter expressing PGE₂ disappearance rate and \(K_{in}(t)\) (ng/mL/h) is a zero-order time-function expressing PGE₂ production rate. \(K_{in}\) is considered as a time-dependent parameter, influenced by carrageenan administration and NSAID concentration (in the periods when administered). To express the action of carrageenan on \(K_{in}\), a stimulation function (named \(\text{stimul}_{\text{PLACEBO}}\) and \(\text{stimul}_{\text{NSAIDs}}\)) was selected as equations 4 and 5 for the placebo and NSAID periods, respectively:

\[
\text{stimul}_{\text{PLACEBO}} = \text{carrag} \times \left( e^{-k_1 \times (t - t_{\text{lag}1})} - e^{-k_2 \times (t - t_{\text{lag}1})} \right)
\]

\[
\text{stimul}_{\text{NSAIDs}} = \text{carrag} \times \left( e^{-k_1 \times (t - t_{\text{lag}2})} - e^{-k_2 \times (t - t_{\text{lag}2})} \right)
\]

Where \(k_1\) and \(k_2\) are the first-order rate constants (1/h) describing the time development of the carrageenan stimulation, carrag is a scalar factor, and \(t_{\text{lag}1}\) and \(t_{\text{lag}2}\) represent the delays in the onset of inflammation for the placebo and NSAID periods, respectively. Consequently, \(t_{\text{lag}}\) is the only delay between \(\text{stimul}_{\text{PLACEBO}}\) and \(\text{stimul}_{\text{NSAIDs}}\) function. Equations 4 and 5 assume that the effect of carrageenan stimulation of COX builds up progressively (as reflected by \(k_2\) after injection, then steadily decreases (as reflected by \(k_1\); Lepist & Jusko, 2004).

It was assumed that robenacoxib and ketoprofen suppressed the carrageenan action in exudate through an \(I_{\text{max}}\) function (Lees et al., 2004) of the form (equation 6):

\[
I(t) = 1 - \frac{I_{\text{max}} \times C(t)^n}{IC_{50}^n + C(t)^n}
\]

\(I(t)\) is a time-dependent scalar. \(I_{\text{max}}\) is a scalar fixed to 1, expressing the fact that robenacoxib can totally inhibit carrageenan pro-inflammatory effect. \(IC_{50}\) expresses the NSAID potency against carrageenan effect; \(n\) is the Hill exponent expressing the steepness of the NSAID concentration vs. effect curve. Finally, incorporating equation 4 (placebo) or equations 5 and 6 (NSAID) in the general equation 3, the time development of PGE₂ concentration in exudate was described by equation 7 (placebo) and equation 8 (NSAID):

\[
\frac{d\text{PGE}_2}{dt} = K_{in}(t) - K_{out} \times \text{PGE}_2
\]

The time courses of exudate PGE₂ were modelled simultaneously for placebo and robenacoxib, then placebo and S(+) ketoprofen, as the equations for placebo and NSAIDs share several common parameters in the same cat (\(K_{in}\), \(K_{out}\) and \(\text{carrag}\), \(k_1\) and \(k_2\)). Nine parameters were estimated by the model, namely \(K_{in}\), \(\text{carrag}\), \(k_1\), \(k_2\), \(t_{\text{lag}1}\), \(t_{\text{lag}2}\), \(K_{out}\ IC_{50}\) and \(n\).

Ex vivo generation of serum TxB₂ was used as a surrogate marker of COX-1 activity for PK/PD modelling. The NSAID concentration in the central compartment produced an inhibition of serum TxB₂ synthesis according to the following sigmoid quantification of the assay) relative to \(IC_{50}\) (ng/mL) is the concentration that achieves half of the maximal TxB₂ suppression and \(n\) is the slope of the NSAID concentration-effect curve. In most cats, the serum TxB₂ concentration had drifted below baseline by the end of the period when placebo was administered, as also reported in a previous study (Pelligand et al., 2012b). This drift of baseline throughout the course of the experiment was modelled as (equation 10):

\[
I(C(t)) = I_0(t) - \frac{(I_0 - I_{\text{max}}) \times C(t)^n}{IC_{50}^n + C(t)^n}
\]

Where \(I_0\) (1) is the baseline serum TxB₂ concentration (ng/mL) for an individual cat, \(I_{\text{max}}\) (%) is the percentage of maximal TxB₂ suppression (corresponding to the lower limit of quantification of the assay) relative to \(I_0\). \(IC_{50}\) (ng/mL) is the concentration that achieves half of the maximal TxB₂ suppression and \(n\) is the slope of the NSAID concentration-effect curve. In most cats, the serum TxB₂ concentration had drifted below baseline by the end of the period when placebo was administered, as also reported in a previous study (Pelligand et al., 2012b). This drift of baseline throughout the course of the experiment was modelled as (equation 10):

\[
I(t) = I_0 - d \times t
\]

Where \(d\) represents the slope of the baseline function for an individual cat and \(I_0\) the initial TxB₂ concentration during the treatment period (Ollerstam et al., 2006). The slope was calculated for each cat by linear regression of the serum TxB₂ concentration after placebo administration. As blood samples were collected for 48 h during the ketoprofen period but only for 24 h after placebo and robenacoxib dosing, the drift was not applied between 24 and 48 h (equation 11):

\[
I_0(t \geq 24\ h) = I_0 - d \times 24
\]
maximal effect ($I_{\text{max}}$), potency (IC\textsubscript{50}) and steepness of the NSAIDs concentration/effect relationship (n). An average curve for COX-1 and COX-2 was fitted to the individual curves previously simulated (naive pooled approach) using the same Hill equation (Giraudel\textsuperscript{*} et al., 2005b; Pelligand et al., 2012b). The corresponding average parameter values (IC\textsubscript{50} and n) and 95% confidence intervals were derived to calculate the selectivity indices to describe the relative in vivo selectivity. Finally, the predicted percentage of COX-1 inhibition was calculated for 50%, 80%, 95% and 99% inhibition of COX-2.

Statistical analysis

Figures and potency curve fitting were computed using Prism version 5 (GraphPad, La Jolla, CA, USA). Statistics were performed with PASW Statistics (version 17, IBM, New York, USA) using a linear mixed model for PGE\textsubscript{2} and TxB\textsubscript{2}. Treatment, time and treatment-time interaction were entered as fixed effects, and cat was entered as a random effect. Time was nested within treatment and cat. A first-order autoregressive covariance structure (AR1) was used (Littell et al., 1998; Kristensen & Hansen, 2004). The normality assumption of the residuals was assessed by visual inspection and was verified after a log transformation of exudate PGE\textsubscript{2} and serum TxB\textsubscript{2}. All reported P values are two-tailed, with statistical significance defined as $P < 0.05$. In the post hoc tests, multiple analyses were corrected using the Bonferroni method. Arithmetic, geometric and harmonic means are presented (in tables only) as mean ± SD, mean [95% Confidence Interval] and mean ± pseudoSD (obtained by the jackknife method), respectively (Lam et al., 1985).

RESULTS

Pharmacokinetics

Pharmacokinetic parameters for plasma ketoprofen and blood robenacoxib concentrations are summarized in Table 1. The plasma concentration/time curve of ketoprofen was best described by a bicompartamental model with first-order absorption for the S(+) enantiomer and a monocompartmental model with first-order absorption for the R(−) enantiomer (Fig. 2). Peak plasma concentrations were 4306 ng/mL for S(+) ketoprofen ($T_{\text{max}} = 0.53$ h) and 3787 ng/mL for R(−) ketoprofen ($T_{\text{max}} = 0.25$ h). Apparent clearances (CL/F) were 0.114 L/kg/h for S(+) ketoprofen and 0.325 L/kg/h for R(−) ketoprofen. Terminal elimination half-life was longer for S(+) ketoprofen ($t_{1/2} = 1.62$ h, MRT = 1.7 h) than for R(−) ketoprofen ($t_{1/2} = 0.44$ h, MRT = 0.7 h).

The blood concentration-time curve of robenacoxib was best described by a bicompartamental model with first-order absorption (Fig. 3). Peak plasma concentration of 1313 ng/mL was reached after 0.9 h, and the mean absorption $t_{\text{lag}}$ was 0.05 h. Apparent blood robenacoxib clearance was moderate (0.684 L/kg/h; Toutain & Bousquet-Melou, 2004) and elimination half-life was 1.13 h.

Pharmacokinetic parameters for exudate are summarized in Table 2. The exudate ketoprofen enantiomer concentrations followed a bi-exponential decay (Fig. 2). Harmonic mean penetration half-lives of S(+) and R(−) ketoprofen in exudate were 2.93 and 2.06 h, respectively. Maximum exudate concentrations were reached at 7.9 and 6.0 h after injection for S(+) and R(−) ketoprofen, respectively. The mean peak exudate concentration of S(+) ketoprofen was 169 ng/mL and that of R(−) ketoprofen was 44 ng/mL. Elimination half-lives from tissue cages were 25.9 h for S(+) and 22.5 h for R(−) ketoprofen, accounting for correspondingly long MRTs of 35.9 and 36.2 h.

Exudate robenacoxib concentration followed a bi-exponential decay (Figs 3 and 4). One cat (D2) had peak robenacoxib exudate concentration (351 ng/mL) that was approximately fourfold higher than the average $C_{\text{max}}$ value observed in the other seven cats. However, its exudate concentrations were similar to those observed in the other seven cats by the 12th hour postdose. As a similar inconsistency was not observed when this cat was administered ketoprofen and because this

<table>
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<th>Parameters</th>
<th>Unit</th>
<th>S(+) Ketoprofen</th>
<th>R(−) Ketoprofen</th>
<th>Robenacoxib</th>
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<td>Mean*</td>
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<tr>
<td>$V_{z,F}$</td>
<td>L/kg</td>
<td>0.308</td>
<td>[0.192–0.495]</td>
<td>0.222</td>
</tr>
<tr>
<td>CL\textsubscript{F}</td>
<td>L/h/kg</td>
<td>0.114</td>
<td>[0.092–0.142]</td>
<td>0.325</td>
</tr>
</tbody>
</table>

* $T_{\text{max}}$ and MRT are presented as arithmetic mean ± SD, half-lives presented as harmonic means with pseudo-SD estimated by the jackknife method. All other parameters are presented as geometric mean [95% CI of the mean]. Calculation methods are given in the text. $T_{\text{max}}$: time of maximal concentration, $C_{\text{max}}$: maximal concentration, AUC\textsubscript{0–inf}: area under concentration vs. time curve extrapolated to infinity, $z$ slope of the drug elimination phase and $t_{1/2}$ corresponding elimination half-life, $V_{z,F}$: volume of the central compartment scaled by bioavailability, F, CL\textsubscript{F}: body clearance scaled by bioavailability.
cat did not behave as an outlier during the pharmacodynamics or blood level PK component of this investigation, it was assumed that these high initial robenacoxib concentrations were a function of experimental error. Accordingly, cat D2 was excluded from the robenacoxib exudate evaluations. However, it should be noted that in the absence of a confirmed source of this error, it is impossible to exclude the possibility that the exudate profiles associated with cat D2 reflect an idiosyncrasy that may exist in a subpopulation of cats. That said, the maximum robenacoxib concentration for the seven other cats was 85.2 ng/mL, attained at 8.1 h after dosing. Harmonic mean penetration half-life of robenacoxib in inflammatory exudate was 4.9 h. Exudate elimination half-life and MRT were 41.5 and 45.7 h, respectively.

Pharmacodynamics

Both ketoprofen and robenacoxib reduced exudate PGE$_2$ concentrations significantly between 6 and 36 h (Fig. 5). Maximum PGE$_2$ inhibition, at 9 h, was 92.1% for robenacoxib and 90.9% for ketoprofen.

Maximal TxB$_2$ suppression with robenacoxib was 51.2% at 2 h, and this was the only time when the effect of robenacoxib was significantly different from placebo (Fig. 6). TxB$_2$ had returned to placebo level at 3 h. With ketoprofen, serum TxB$_2$ inhibition occurred rapidly, commencing 4 min after injection (97.1%) and suppression was maximal (97.9%) at 1 h (Fig. 6). Compared with placebo, ketoprofen significantly suppressed serum TxB$_2$ between 4 min and 24 h. Serum TxB$_2$ was 11.8%
PK/PD analysis

For COX-2 inhibition, the PK/PD model for the estimation of pharmacodynamic parameters gave good results in six of eight cats for both S(+)-ketoprofen and robenacoxib. In two cats, the model did not converge, because exudate PGE₂ concentrations were reduced below the limit of quantification of the assay or did not recover to the levels in the placebo group within 120 h. Means of individual estimates of the pharmacodynamic COX-2 parameters for the carrageenan model, and after administration of ketoprofen and robenacoxib, are presented in Table 3. The geometric mean COX-2 IC₅₀ was 44.7 ng/mL (0.14 μM) for robenacoxib and 45.0 ng/mL (0.18 μM) for S(+) ketoprofen.

PK/PD modelling for COX-1 was successful in all animals with robenacoxib and in six of eight cats with S(+) ketoprofen. For the latter, in two cases, the number of blood samples was too low to allow bi-compartmental fitting of plasma concentrations and thus prevented PK/PD modelling. Individual geometric mean IC₅₀ COX-1 was 29.51 ng/mL (1.31 μM) for robenacoxib and 0.17 ng/mL (0.67 nM) for S(+) ketoprofen (Table 4). Iₘ₅ₐₓ was 97.3% for S(+) ketoprofen and 96.8% for robenacoxib.

Individual concentration-effect curves were simulated using the pharmacodynamic parameters aforementioned. Average pharmacodynamic parameters (Iₘ₅ₐₓ, IC₅₀ and μ) for S(+) ketoprofen and robenacoxib for the inhibition of COX-1 in serum and COX-2 in exudate were calculated by naive pooled data analysis (Table 5 and Fig. 7). The concentration/effect curves for COX-1 required re-scaling to a maximal effect of 100%. The IC₅₀ values for COX-1 were 0.45 and 2557 ng/mL for S(+) ketoprofen and robenacoxib, respectively.

Fig. 4. Individual exudate robenacoxib concentration (ng/mL) versus time (h) profiles after after subcutaneous administration of a 2 mg/kg dose.

Fig. 5. Exudate PGE₂ concentration (ng/mL) versus time (h) profiles after carrageenan injection and placebo, racemic ketoprofen (2 mg/kg total dose) and robenacoxib (2 mg/kg) subcutaneous administration. PK/PD modelling is relevant to the time-response profile as a whole rather than to the response at sampling times taken separately and therefore values are presented as mean ± SEM. Statistical comparison of effect of treatment versus placebo (*P < 0.05) at different times (linear mixed effect model).

Fig. 6. Serum TxB₂ concentration (ng/mL) versus time (h) profile after placebo, racemic ketoprofen (2 mg/kg total dose) and robenacoxib (2 mg/kg) subcutaneous administration. PK/PD modelling is relevant to the time-response profile as a whole rather than to the response at sampling times taken separately and therefore values are presented as mean ± SEM. Statistical comparison of effect of ketoprofen versus placebo (*P < 0.05) and robenacoxib versus placebo (£= P < 0.05) at different times (linear mixed effect model).
Table 3. Individual pharmacodynamic parameters describing the inhibitory effect of robenacoxib (2 mg/kg) and ketoprofen (2 mg/kg racemate) on exudate PGE₂ production after subcutaneous administration

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Unit</th>
<th>Robenacoxib*</th>
<th>Ketoprofen*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>95% CI or [range]</td>
<td>Mean</td>
</tr>
<tr>
<td>Kᵣᵢṇ</td>
<td>ng/mL/h</td>
<td>0.28</td>
<td>0.01–0.79</td>
</tr>
<tr>
<td>Carrag</td>
<td>no unit</td>
<td>33.3</td>
<td>11.0–100.9</td>
</tr>
<tr>
<td>k₁</td>
<td>/h</td>
<td>0.21</td>
<td>0.08–0.54</td>
</tr>
<tr>
<td>k₂</td>
<td>/h</td>
<td>0.05</td>
<td>0.03–0.10</td>
</tr>
<tr>
<td>N</td>
<td>no unit</td>
<td>2.0</td>
<td>1.09–3.78</td>
</tr>
<tr>
<td>IC₅₀</td>
<td>ng/mL</td>
<td>44.7</td>
<td>16.9–118.2</td>
</tr>
<tr>
<td>Kᵣᵢᵡ</td>
<td>/h</td>
<td>0.12</td>
<td>0.04–0.39</td>
</tr>
<tr>
<td>tᵢᵣᵢₙ₁</td>
<td>placebo</td>
<td>h</td>
<td>0.5</td>
</tr>
<tr>
<td>tᵢᵣᵢₙ₂</td>
<td>NSAID</td>
<td>h</td>
<td>5.4</td>
</tr>
</tbody>
</table>

An indirect response model including nine estimated parameters was computed.

*The results from two of eight cats receiving ketoprofen were excluded from the calculation of the mean because the inhibition of exudate PGE₂ never recovered below 50% of placebo PGE₂. The NSAIDs function (intercept at time 0 taking into account baseline drift in TxB₂ concentrations observed after placebo dosing. Data are presented as geometric mean with 95% CI of the mean except tᵢᵣᵢₙ₁ and tᵢᵣᵢₙ₂. Data are reported as geometric mean with 95% CI of the mean except tᵢᵣᵢₙ₁ (arithmetic mean, [range]).

Table 4. Individual pharmacodynamic parameters describing the inhibitory effect of robenacoxib (2 mg/kg) and ketoprofen (2 mg/kg racemate) on serum TxB₂ production (COX-1 activity) after subcutaneous administration

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Unit</th>
<th>Robenacoxib</th>
<th>Ketoprofen*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>95% CI</td>
<td>Mean</td>
</tr>
<tr>
<td>I₀</td>
<td>ng/mL</td>
<td>174</td>
<td>115–262</td>
</tr>
<tr>
<td>IC₅₀</td>
<td>ng/mL</td>
<td>2951</td>
<td>1498–5815</td>
</tr>
<tr>
<td>N</td>
<td>no unit</td>
<td>1.01</td>
<td>0.65–1.58</td>
</tr>
</tbody>
</table>

*The results from two of eight cats receiving ketoprofen were excluded, as the number of samples was too low to fit a biexponential model to the blood concentration time profile. Data were fitted using a sigmoid Iₘₐₓ model for robenacoxib (eight cats) and ketoprofen (six cats). Iₘₐₓ is the percentage of maximal suppression of TxB₂ (corresponding to the lower limit of quantification of the assay) relative to I₀(t). I₀ is the fitted value of intercept at time 0 taking into account baseline drift in TxB₂ concentrations observed after placebo dosing. Data are presented as geometric mean and 95% CI of the mean for I₀, IC₅₀ and n.

Table 5. Average maximal effect (Iₘₐₓ), potency (IC₅₀) and slope (n) of S(+) ketoprofen and robenacoxib for ex vivo inhibition of COX-1 in serum and in vivo inhibition of COX-2 in exudate

<table>
<thead>
<tr>
<th>PD COX parameters (units)</th>
<th>Iₘₐₓ (%)</th>
<th>IC₅₀ (ng/mL)</th>
<th>[95% CI]</th>
<th>n (no unit)</th>
<th>[95% CI]</th>
</tr>
</thead>
<tbody>
<tr>
<td>COX-1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ketoprofen</td>
<td>97.3</td>
<td>0.45</td>
<td>[0.32–0.65]</td>
<td>0.66</td>
<td>[0.51–0.81]</td>
</tr>
<tr>
<td>Robenacoxib</td>
<td>96.8</td>
<td>2557</td>
<td>[2291–2818]</td>
<td>0.87</td>
<td>[0.80–0.94]</td>
</tr>
<tr>
<td>COX-2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ketoprofen</td>
<td>100.0</td>
<td>48.5</td>
<td>[41.6–56.6]</td>
<td>1.04</td>
<td>[0.89–1.19]</td>
</tr>
<tr>
<td>Robenacoxib</td>
<td>100.0</td>
<td>38.2</td>
<td>[33.9–42.7]</td>
<td>1.46</td>
<td>[1.22–1.70]</td>
</tr>
</tbody>
</table>

Reported parameters and bounds of the 95% confidence interval [95% CI] were calculated by naïve pooled data analysis (Giraudel et al., 2005a,b). An average curve was fitted with a sigmoid Iₘₐₓ model to all simulated curves (n = 8 cats for robenacoxib COX-1 and six cats otherwise) as if they were data from a single individual.

(+)-ketoprofen and robenacoxib and the slopes (n) were 0.66 and 0.87, respectively. Corresponding IC₅₀ values for COX-2 were 48.5 and 38.2 ng/mL for S(+) ketoprofen and robenacoxib, respectively, and corresponding slopes were 1.04 and 1.46.

Three categories of indices were used to describe the selectivity of robenacoxib, determined by simultaneous fitting of individual percentage inhibition values from COX-1 and COX-2 assays (Table 6). The IC₅₀/COX-1/IC₅₀/COX-2 ratio was 1:107 for S(+) ketoprofen and 66.9:1 for robenacoxib. The selectivity of robenacoxib for COX-2 was confirmed at virtually maximal inhibition, as IC₉₀/COX-1/IC₉₀/COX-2 was 585:1. The IC₂₀ COX-1/IC₅₀ COX-2 ratio was 1:3260 for S(+) ketoprofen and 1:4:1 for robenacoxib. Predicted percentage inhibitions of COX-1 vs. COX-2 are illustrated in Fig. 8; the inhibition of COX-1 by

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**DISCUSSION**

**S(+) ketoprofen** was the predominant enantiomer in the cat, as previously reported for the dog, rat and horse (Foster & Jamali, 1988; Delatour et al., 1993; Landoni & Lees, 1995a). Chiral inversion of R(−) to S(+) ketoprofen occurs in the liver, so that the R(−) enantiomer, although itself of very low potency, is a pro-drug. Therefore, the apparent clearance of R(−) ketoprofen incorporates both elimination and inversion to the S(+) enantiomer. Consequently, the drug input for S(+) ketoprofen comprises both the administered drug and S(+) ketoprofen formed by chiral inversion. The inversion rate has been calculated in the cat by separate administration of each enantiomer (Castro et al., 1988; Delatour et al., 2000; Lees et al., 2003). Simultaneous enantiomer pharmacokinetic modelling was not possible, as the inversion rate could not be identified from the data of the present study. This study confirmed the short half-life of both ketoprofen enantiomers in the cat. The pharmacokinetics of robenacoxib after subcutaneous administration was also consistent with the findings from previous studies (Pelligand et al., 2012b; King et al., 2013), with a short elimination half-life (1.1 h).

Despite having short elimination half-lives in blood, ketoprofen and robenacoxib demonstrated marked negative hysteresis. Both drugs suppressed exudate PGE2 significantly for up to 36 h. The likely explanation is accumulation of drugs in and slow clearance from the tissue cage. It would have been relevant to test this hypothesis by directly injecting the test article into the tissue cages. The IC_{50} COX-2 for robenacoxib was somewhat higher at 38.2 ng/mL (0.117 μM) in the present study compared with 14.1 ng/mL (0.043 μM) reported in the study described by Pelligand et al. (2012b). For COX-1, the difference for robenacoxib between the two studies was minimal, with IC_{50} COX-1 of 2557 ng/mL (7.81 μM) in the present study and 2416 ng/mL (7.38 μM) in the previous investigation.

The persistence and duration of effect in exudate of ketoprofen enantiomers were similarly long as for robenacoxib. For 2-arylpropionates in general, and for ketoprofen in particular, COX inhibition activity resides almost exclusively with the S(+) enantiomer (Hayball et al., 1992; Suesa et al., 1993; Landoni et al., 1996) in several species including the cat. It is, indeed, probable that COX inhibition in the cat after R(−) ketoprofen administration is attributable solely to the S(+) enantiomer.
formed in vivo by chiral inversion (Lees et al., 2003). It was therefore justified, in this study, to conduct PK/PD modelling solely on S(+) ketoprofen concentration. The IC₅₀ COX-2 for S(+) ketoprofen of 48.5 ng/mL (0.191 μM) was very similar to the IC₅₀ for robenacoxib in the present study (38.2 ng/mL) but was lower than the IC₅₀ reported by Schmid et al. (2010) in vitro whole blood assays: 119.9 ng/mL (0.472 μM). In serum, the ex vivo IC₅₀ for COX-1 in the present study was 0.454 ng/mL (0.0018 μM) which was lower than the IC₅₀ of 5.92 ng/mL (0.023 μM) reported by Schmid et al. (2010) in vitro assays. Interlaboratory differences in experimental methodology (ex vivo vs. in vitro) and differences in modelling techniques are well recognized as the basis for differing results, even of this relatively high magnitude (Warner et al., 1999). In consequence, we report an IC₅₀ COX-1/IC₅₀ COX-2 ratio of 1:107, which is lower than that obtained by Schmid et al. of 1:20. Despite these numerical differences, both studies confirm that ketoprofen is COX-1 selective in the cat. The time-course of inhibition of TxB₂ with ketoprofen was similar to that reported after intravenous administration of 2 mg/kg racemic ketoprofen (Lees et al., 2003).

The present data indicate that ketoprofen and robenacoxib exhibit similar pattern for distribution to a site of acute inflammation, while possessing opposite selectivities for the inhibition of COX isoforms, ketoprofen for COX-1 and robenacoxib for COX-2. As discussed by (Brune & Furst, 2007), the first-generation selective COX-2 inhibitors (sulphonamides and methylsulphones) combined reduced gastrointestinal toxicity with prolonged inhibition of constitutively expressed COX-2 in the vascular wall and kidney. This may explain, at least partially, the reported toxicities of these COXibs with long terminal half-lives and large volumes of distribution. It is therefore likely that tissue selectivity is a potential advantage of second-generation COXibs (carboxylic acids) with shorter elimination half-lives. If these drugs exert only a short duration of action on constitutively expressed COX-2 in the central pharmacokinetic compartment, this might provide a higher safety profile, for example for cardiovascular and renal side-effects. As developed in our laboratory, the tissue cage model has allowed investigation of the distribution of robenacoxib and ketoprofen (selected for this study for both their differing COX inhibition profiles and long durations of action despite short half-lives in the central pharmacokinetic compartment) to a site of acute inflammation. It may be regarded as an appropriate model to further our understanding of other carboxylic acid NSAIDs, with similar chemical structures and pharmacokinetic profiles, such as lumiracoxib (COX-2 selective) and diclofenac (COX nonselective; Fig. 8; Brune & Furst, 2007).

It should, however, be noted that all tissue cage models are ‘model dependent’, in that drug diffusion into and from exudate in the cage is influenced by tissue cage geometry (including surface area), a lack of physiological drainage as for the synovial fluid lymph drainage, as well as drug molecule properties, including protein binding, pKa and lipid solubility. Therefore, tissue cage models cannot mimic either accurately or quantitatively all clinical circumstances. Nevertheless, it is of interest to note that lumiracoxib accumulated in inflamed joints in humans and its concentration was maintained in excess of plasma concentrations for up to 18 h after dosing (Scott et al., 2004). Similarly, ketoprofen penetrated readily into acutely inflamed joints of the horse. At one h after dosing the concentration in synovial fluid was six times higher in inflamed compared with noninflamed joints (Owens et al., 1994). On the other hand, concentrations of etoricoxib (a coxib of the sulphonamide group) in wound fluids did not exceed plasma concentrations after pre-emptive administration before hip surgery (Renner et al., 2010, 2012).

It is unlikely that the slow clearance of ketoprofen and robenacoxib from tissue cages was limited by passive diffusion. This is suggested by serum and exudate clearance data. For creatinine, an endogenous, nonprotein bound small molecule, a MRTexudate/MRTserum ratio of 3:6:1 was obtained by Pelligand et al. (2012a). In contrast, robenacoxib and S(+) ketoprofen MRTexudate/MRTblood ratios in the present study were substantially higher, 24.9:1 and 20.4:1, respectively. These high ratios are explained by two factors, slow drug clearance from the tissue cages and short half-lives in plasma. Despite the use of tissue cages of different geometry (spherical polypropylene cages instead of silicon cylinders similar to the feline tissue cages), previous workers showed that the ketoprofen MRT ratio was also high (11.5:1) in the goat (Arifah et al., 2003) and in the calf (10.6:1; Landoni & Lees, 1995b) though not in the horse (2.9:1). Moreover, other tissue cage investigations demonstrated that not all COXibs are tissue selective: the MRTexudate/MRTblood ratio for firocoxib in the dog (a methylsulphone related to rofecoxib) was 1.06:1 and similar to meloxicam 1.08:1 (P. Lees, unpublished data).

The binding of drugs to and slow release from a component of the inflammatory process, such as protein or a specific cell population, could account for these differing results for NSAIDs (Pelligand et al., 2012b). For example, the search for the ideal radiolabelled marker for imaging COX-2 expression revealed that a radioiodinated derivative of lumiracoxib had a higher affinity and in vitro cell uptake for COX-2 induced macrophages than normal macrophages (Kuge et al., 2009). A similar mechanism might explain the slow clearance of robenacoxib from exudate.

The prolonged plasma half-life of the sulphonamide COXibs (celecoxib, etoricoxib and valdecoxib) is explained by both slow clearance and relatively high volume of distribution. In contrast, the volume of distribution of carboxylic acid COXibs is very small compared with other classes of COXibs. Thus, lumiracoxib steady-state volume of distribution was 9 L in humans (0.13 L/kg for a 70 kg person, Mysler, 2004) and the distribution volume for robenacoxib was likewise low, 0.19 L/kg in the cat and 0.24 L/kg in the dog (Jung et al., 2009; Pelligand et al., 2012b).

The sulphonamide moiety of a radioiodinated derivative of celecoxib had a high affinity for carbonic anhydrase, and this could explain both the preferential distribution into rat erythrocytes (88%) and slow clearance from blood (Boddy et al.,...
1989; Kuge et al., 2006). Substitution of the sulphamamide moiety to a methylsulphone moiety decreased erythrocyte binding to 18% and increased blood clearance (Kuge et al., 2006).

In conclusion, the present data suggest that, despite a short blood half-life, NSAIDs can have a long-lasting local action, as a consequence of high inflammatory tissue selectivity. However, drug distribution into tissue cage fluid is model dependant and cannot be a precise predictor of penetration time course to other anatomical sites. This will indeed depend on a wide range of factors, including specific tissue blood flow and possibly the degree of acute inflammation. Tissue selectivity might be advantageous for carboxylic acids COXibs and some older NSAIDs such as ketoprofen and diclofenac, as systemic side-effects related to COX-1 and COX-2 inhibition could be reduced, while efficacy in experimental inflammation persists for 24 h or longer after a single dose.

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DECLARATIONS OF INTEREST

L. Pelligand received a CASE award from BBSRC and Novartis Animal Health. P. Lees has acted as a consultant to Novartis Animal Health. J.N. King is an employee of Novartis Animal Health. L. Pelligand received a CASE award from BBSRC and Novartis Animal Health. P. Lees has acted as a consultant to Novartis Animal Health. J.N. King is an employee of Novartis Animal Health.

REFERENCES


