

Use of a pharmacokinetic/pharmacodynamic approach in the cat to determine a dosage regimen for the COX-2 selective drug robenacoxib

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This study investigated the analgesic, anti-inflammatory and antipyretic efficacy of the new COX-2 selective inhibitor robenacoxib in the cat and established pharmacodynamic (PD) parameters for these effects. Robenacoxib, at a dosage of 2 mg/kg administered subcutaneously, was evaluated in a kaolin-induced paw inflammation model in 10 cats, using both clinically relevant endpoints (lameness scoring, locomotion tests) and other indicators of inflammation (body and skin temperature, thermal pain threshold) to establish its pharmacological profile. A pharmacokinetic/pharmacodynamic (PK/PD) modelling approach, based on indirect response models, was used to describe the time course and magnitude of the responses to robenacoxib. All endpoints demonstrated good responsiveness to robenacoxib administration and both the magnitude and time courses of responses were well described by the indirect pharmacodynamic response models. Pharmacokinetic and clinically relevant pharmacodynamic parameters were used to simulate dosage regimens that will assist the planning of clinical trials and the selection of an optimal dosage regimen for robenacoxib in the cat.

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INTRODUCTION

Pharmacokinetic/pharmacodynamic (PK/PD) modelling approaches have proved useful for quantifying drug response as well as characterizing mechanisms of action for nonsteroidal anti-inflammatory drugs (NSAIDs) (Torres-Lopez *et al.*, 1997; Flores-Murrieta *et al.*, 1998; Josa *et al.*, 2001). In animal species of veterinary interest, two groups have demonstrated the usefulness of modelling blood (or plasma) concentration profiles with the time course of NSAID effects and responses (Toutain *et al.*, 1994; Landoni *et al.*, 1995b; Landoni & Lees, 1995; Toutain *et al.*, 2001; Lees, 2003; Lees *et al.*, 2004; Giraudel *et al.*, 2005a,b).

Pharmacokinetic/pharmacodynamic modelling permits *in vivo* determination of the three key PD parameters of a drug, namely efficacy, potency, sensitivity, and duration of response. These parameters usually cannot be derived from classical dose-titration studies, because PK data are usually lacking and interpolation or extrapolation between the doses tested can be

misleading in investigations utilizing a parallel design. On the other hand, testing a single dose may be sufficient to determine an optimal dosage regimen when using PK/PD modelling approaches, and the derived parameters can also be applied to the prediction of the time course of drug response for any formulation, route of administration or dosage regimen, provided corresponding PK data are available (Toutain *et al.*, 2001; Toutain, 2002; Toutain & Lees, 2004).

To generate PD drug parameters that are relevant to subsequent clinical evaluation, it is appropriate to administer the NSAID after rather than before the onset of inflammation, in contrast to the methods usually adopted in rodent models (Josa *et al.*, 2001). The inflammatory response in the selected model should be sufficiently persistent (at least 4 days) to enable monitoring of the offset as well as the onset of response. Moreover, it is necessary to use quantitative, validated, clinically relevant endpoints, both to describe the magnitude and time course of drug response and to document the clinical efficacy of administered drugs.

A recently developed kaolin-induced paw inflammation model in the cat meets all the requirements to undertake PK/PD modelling of NSAIDs in a preclinical study (Giraudel *et al.*, 2005a,b). In this model, several endpoints have been assessed for accuracy and reproducibility as well as clinical relevance (Giraudel *et al.*, 2005a,b). Locomotion tests, body and skin temperature, lameness score and thermal pain threshold testing allowed the analgesic, anti-inflammatory and antipyretic effects of meloxicam to be studied (Giraudel *et al.*, 2005a). Few previous studies have described objective and quantitative measurements of pain or hyperalgesia in the cat (Dixon *et al.*, 2002; Lascelles & Robertson, 2004), but these two criteria are met by the kaolin model of inflammation and the thermal threshold testing device used to monitor hyperalgesia in this model (Giraudel *et al.*, 2005a,b).

Robenacoxib is a new NSAID being developed for the treatment of inflammatory and painful conditions of cats and dogs when administered by subcutaneous (SC) and oral routes. Its pharmacological properties in laboratory animal studies have been described by King *et al.* (2008). Robenacoxib is highly selective for cyclooxygenase (COX)-2 in whole blood assays (Giraudel *et al.*, 2008). The aims of the present study were to quantify in the cat the analgesic, anti-inflammatory and antipyretic effects of robenacoxib in a recently validated kaolin-induced paw inflammation model and to establish *in vivo* key PD parameters for these effects by PK/PD modelling. These parameters were then used to simulate responses to several dosage regimens, the ultimate goal being to assist the selection of a dose of robenacoxib for subsequent assessment in clinical trials.

MATERIALS AND METHODS

Animals

The study was performed in 10 healthy European short-haired cats of both sexes (five males, five females), maintained in a temperature controlled environment (20 ± 2 °C) and either loose-housed in a colony (between experiments) or kept in individual stainless steel cages (during experiments). Weights and ages ranged from 3.1 to 4.1 kg and 1.2 to 2.2 years, respectively. Every evening after the last measurements, the cats were fed commercial dry food [Fit 32 (60 g once a day), Royal Canin SA BP4, Aimargues, France]. The housing and experimental facilities at the National Veterinary School of Toulouse were approved by the French Ministry of Agriculture, and animal care and the conduct of the study was performed in accordance with the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council, 1996). The study was performed in compliance with the Procedures and Principles of Good Clinical Practice (CVMP/VICH/595/98) and with the Guideline for the Conduct of Efficacy Studies for NSAIDs (EMA/CVMP/237/01).

Drugs and chemicals

Ketamine, xylazine and medetomidine were obtained from Merial SAS (Lyon, France), Bayer Pharma (Puteaux, France) and Pfizer Santé Animale (Orsay, France), respectively. Kaolin (hydrated aluminium silicate) was purchased from Sigma-Aldrich (Saint Quentin Fallavier, France). The solution for injection of robenacoxib (20 mg/mL) was obtained from Novartis Animal Health Inc. (CH-4058 Basel, Switzerland) and stored at 4 °C.

Animal preparation and inflammation induction

Animal preparation and inflammation induction were performed using standardized procedures (Giraudel *et al.*, 2005a,b). Five days prior to inflammation induction, each cat was anaesthetized with xylazine (1 mg/kg, SC) and ketamine [5 mg/kg intravenously (IV)] and a central venous catheter with 17 cm long proximal extension (V-PUM-401J-V8-UQ, Cook Veterinary Products Inc, Bloomington, IN, USA) was placed in a jugular vein. Both hind paws were clipped from the digits up to the hock joint and marked for skin temperature and withdrawal time measurements.

Baseline measurements for all endpoints were performed 3 days before kaolin injection. On the day of inflammation induction (day 0), each cat was anaesthetized with medetomidine and ketamine [intramuscular injections (IM 80 µg/kg b.w. and 5 mg/kg b.w., respectively)]. A volume of 1.75 mL of 25% (w/w) sterile suspension of kaolin (containing between 500 and 525 mg kaolin) was injected SC under aseptic conditions into the plantar surface of the right paw (Giraudel *et al.*, 2005b).

Endpoint measurements

Endpoints were recorded using standardized procedures by two trained operators. Measurements were always recorded in the same sequence. The repeatability and reproducibility and methods for all measurements except pain scoring were as described previously (Giraudel *et al.*, 2005b). Briefly, body (rectal) temperature was recorded using an electronic thermometer (ARTSANA, Ref.00.23222.12.00, Centravet, Toulouse, France). The cat was then allowed to walk freely in the study room and gait was scored with a numerical rating scale (NRS) as described by Giraudel *et al.* (2005b).

Locomotion was quantified as the time required by the cat to perform a series of tests [ascending or descending a wooden staircase and creeping under a grid]. To take account of baseline (day-3) and control (pretreatment, i.e. after kaolin injection on Day 0 and just before robenacoxib administration) values, the three locomotion times were transformed into locomotion scores (descending, climbing and creeping scores) and expressed as percentages using the equation:

$$R = \left[1 - \frac{MT - CT}{BT - CT} \right] \times 100 \quad (1)$$

where MT (sec) is the locomotion time after robenacoxib administration, CT (sec) the locomotion time just before injection of robenacoxib (control value) and BT (sec) the mean locomotion time recorded before kaolin injection (baseline value).

Hind paw skin temperature was measured using an infrared thermometer (Raynger[®] MX4[™] Raytek[®], Fisher Bioblock Scientific, Illkirch, France) and recorded on both paws; the difference between the temperature of the injected and the control paw was determined.

Pain was evaluated as the time required by the cat to withdraw its paw after stimulation with the radiant heat emitted from an analgesia meter (Model 390, IITC Inc./Life Science, Woodland Hills, CA, USA). The animal was confined for a few minutes in a Plexiglas chamber adapted to the size of the cat, and placed on top of the glass panel of the device. This method avoided animal restraint and delegated control of the duration of the stimulus to the cat. The radiant heat was a beam of focused light of fixed intensity (20% of maximal intensity), and stimulation commenced when the cat was accurately positioned (close contact of the paw with the glass panel). The timer was stopped when the animal started to withdraw its paw. Only a clear withdrawal or lateral movement of the paw was accepted as an accurate cut-off point and the test was repeated if the response of the cat was not clear-cut. Four measurements were performed on the test paw and two on the control paw at each recording time. Values for the test paw were ranked and the average of the second and third values was used as the value for the withdrawal time. This average value was then transformed into a pain score (%) using an equivalent of Eqn 1.

Study design

The cats were allocated to five groups, each with two animals. During each 2-week period, the right paw of the two cats was injected with 500 mg kaolin and both cats were administered 2 mg/kg robenacoxib SC 47 h after kaolin injection (day 2). The injection site was located on the dorsal line close to the last

thoracic vertebrae. Blood samples (1 mL) were taken before and 5, 15, 30, 45, 60 min and 2, 4, 6, 8, 12 and 23 h after robenacoxib administration. Blood samples were collected in polyethylene tubes containing EDTAK2 and frozen at -20°C until analysed for robenacoxib concentrations by HPLC-UV and LC-MS. Endpoints were measured before (day-3) and daily up to 4 days after kaolin injection (day 1 to 4) (Fig. 1). On day 2, measurements were performed before and 0.5, 1.5, 2.5, 3.5, 4.5, 6, 8, 10 and 12 h after drug administration, each series lasting approximately 25 min. At the end of the study, no animal exhibited any persisting clinical sequelae. All cats were re-homed.

Analysis of robenacoxib in blood

A sensitive GLP validated analytical method using HPLC-UV and LC-MS was used (Jung *et al.*, 2008). Briefly, the method involved an initial analysis of the unknown specimen by HPLC-UV, 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 HPLC-UV method, some samples were diluted, in order not to exceed a concentration of 100 ng/mL in the LC-MS method. The limit of quantification (LOQ) was 3 ng/mL.

Data analysis

Pharmacokinetic and PK/PD modelling was performed by least-squares regression analysis using WinNonlin Professional software (WinNonlin[®], Version 4.0.1, Pharsight Corporation, Mountain View, CA, USA).

Robenacoxib blood concentrations were fitted for each cat using an equation corresponding to a two-compartmental model with first order absorption:

$$C(t) = -(Y_1 + Y_2) \exp(-ka \times t) + Y_1 \exp(-\lambda_1 \times t) + Y_2 \exp(-\lambda_2 \times t) \quad (2)$$

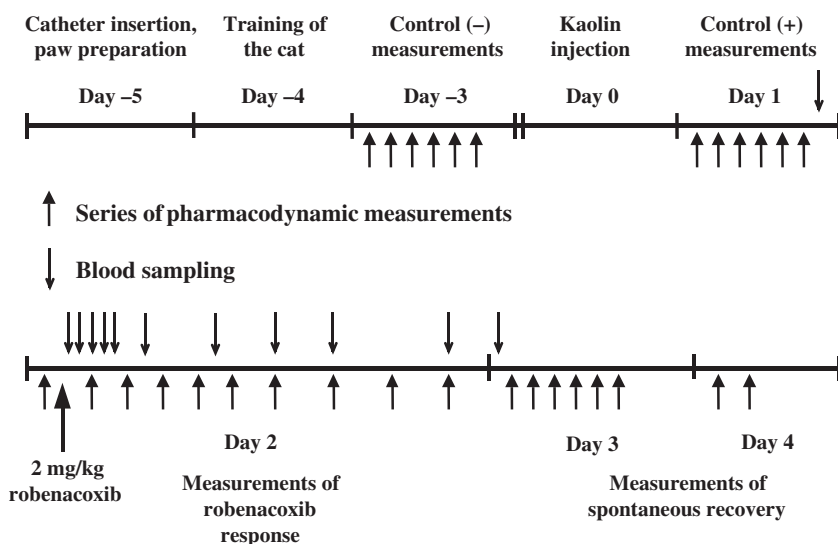


Fig. 1. Time schedule for pharmacodynamic measurements and blood sampling. Robenacoxib was administered at 10:00 a.m., 47 h after kaolin injection. Recording of endpoint values was performed at 10:00 a.m. on days with only one series of measurements, from 9:00 a.m. to 4:00 p.m. when six series of measurements were undertaken, from 8:30 a.m. to 9:30 p.m. on day 2 and at 10:00 a.m. and 12:00 a.m. on day 4.

where $C(t)$ (ng/mL) is the robenacoxib blood concentration at time t , Y_1 , Y_2 (ng/mL) are the coefficients of the exponential terms, λ_1 , λ_2 (h^{-1}) the exponents of the exponential terms and ka (h^{-1}) is the first-order rate constant of absorption.

The data were weighted by the inverse of the squared-fitted value and goodness of fit was determined using the Akaike Information Criterion (AIC) (Yamaoka *et al.*, 1978) and by visual inspection of the fittings and the residuals. Individual animal PK parameters of robenacoxib were then used as constants in the integrated PK/PD model.

The PK/PD relationships were described using indirect pharmacodynamic response models (Dayneka *et al.*, 1993). In these models, the measured response (R) is assumed to result from the factors controlling either the input or the dissipation of the measured response:

$$dR/dt = K_{in} - K_{out}R \quad (3)$$

where dR/dt is the rate of change of the response over time, K_{in} represents the zero-order rate constant for production of the response and K_{out} is the first-order rate constant for loss of the response.

The drug response is considered indirect because it results from physiological processes that are either stimulated or inhibited by the drug. For all endpoints except body temperature, the relevant principal mechanism to consider for a NSAID is inhibition of COX isoenzymes. The drug effect therefore consists of a decrease in synthesis of inflammatory mediators (for example COX-2 derived PGE₂ and/or related prostanoids, although inhibition of other enzymes and mediators is not precluded). The relationship with blood concentration was therefore based on this effect and not on the decrease in the observed response.

For skin temperature difference (the difference between the temperature of the inflamed paw and the control paw), lameness and pain scores and locomotion scores (corresponding to descending, climbing and creeping times) data were described with the following model:

$$dR/dt = K_{in} \left[1 - \frac{I_{max} \times C_{(t)}^n}{IC_{50}^n + C_{(t)}^n} \right] - K_{out}R \quad (4)$$

where K_{in} (units of $^{\circ}\text{C}/\text{h}$, $\%/\text{h}^{-1}$ or h^{-1}) represents the zero-order rate constant for production of the inflammatory response, K_{out} (h^{-1}) the first-order rate constant for dissipation of the inflammation and/or the hyperalgesia; the drug effect was described with a Hill equation in which $C_{(t)}$ (ng/mL) is the robenacoxib blood concentration at time t , IC_{50} (ng/mL) the robenacoxib blood concentration producing half the maximum drug effect (i.e., half I_{max}), I_{max} the maximum possible inhibition and n the exponent expressing the sigmoidicity of the robenacoxib concentration-effect relationship.

The control value for the response (R_0) corresponds to the steady inflammation plateau achieved two days after kaolin injection; it is determined by both K_{in} and K_{out} with:

$$R_0 = \frac{K_{in}}{K_{out}} \quad (5)$$

For the antipyretic effect, another indirect response model was used to account for the effect of robenacoxib on body temperature (Toutain *et al.*, 2001; Giraudel *et al.*, 2005a,b). To achieve a rapid temperature decrease, heat loss is enhanced and the antipyretic effect of NSAIDs, although resulting primarily from a decrease in PGE₂ synthesis, can therefore be described as the consequence of a stimulation of the factors controlling heat loss as:

$$dR/dt = K_{out}R_0 - K_{out} \left[1 + \frac{\left(\frac{R_n}{R_{max}} - 1 \right) \times C_{(t)}^n}{SC_{50}^n + C_{(t)}^n} \right] R \quad (6)$$

where K_{out} (h^{-1}) represents the first-order rate constant for heat loss, R_0 ($^{\circ}\text{C}$) the steady body temperature before robenacoxib administration on day 2, R_{max} ($^{\circ}\text{C}$) the maximum response attributed to the drug (i.e., the minimum body temperature predicted after robenacoxib administration) and SC_{50} (ng/mL) the robenacoxib blood concentration producing half the maximum stimulation of heat loss; $C_{(t)}$, R , and n are as described in Eqn 4.

Using mean PK and PD parameters, simulations were performed to predict the time course of robenacoxib response on each endpoint for doses ranging from 0.01 to 10 000 mg/kg. Because the distribution of individual PK and PD parameters was close to log-normality, the geometric mean was calculated. Whereas data from all individual animals were used to compute mean PK parameters, only cats with acceptable PD fittings were taken into account for the calculation of average PD parameters.

For each simulation, a summary parameter, the average drug response, was used to characterize the time course of each endpoint following robenacoxib administration. All drug effects were considered to have waned 12 h after robenacoxib administration and all calculations were therefore performed by considering the 12-h period following SC injection of the drug.

Simulated average drug responses were calculated for all endpoints as follows:

$$E = \frac{AUR_0 - AUR_X}{12} \quad (7)$$

where E ($^{\circ}\text{C}$, $\%$ or without unit for the lameness score) is the average drug response over the first 12 h after robenacoxib dose, AUR_0 ($^{\circ}\text{C}\cdot\text{h}$, $\% \cdot \text{h}$ or h) is the area under the time-response profile for the same time period and in the absence of robenacoxib administration and AUR_X ($^{\circ}\text{C}\cdot\text{h}$, $\% \cdot \text{h}$ or h) is the same area but after administration of X mg/kg robenacoxib.

The average drug response was then expressed as a percentage of the maximum possible average drug response i.e., the response obtainable for a very high SC dose of robenacoxib (e.g. 100 mg/kg) (Fig. 2). The 40%, 50% and 60% effective doses (ED_{40} , ED_{50} , ED_{60}) were also calculated for each endpoint.

From these ED_{50} s, potencies for drug response were derived with the following equation:

$$EC_{50} = \frac{F \times ED_{50}}{Cl} \quad (8)$$

where ED_{50} (mg/kg/day) is the dose to be administered *in vivo* to give, in steady-state conditions, an average concentration over

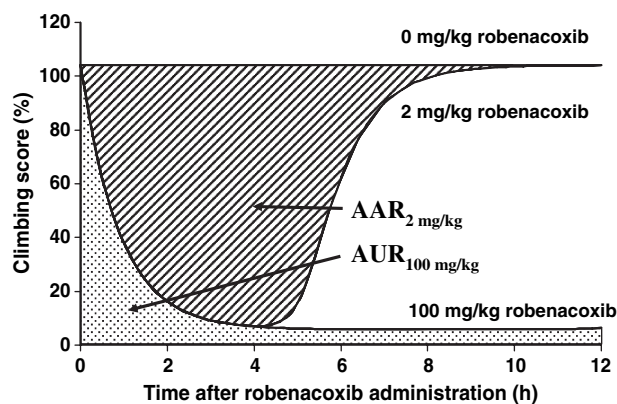


Fig. 2. Time course of the simulated mean climbing score after injection of 500 mg kaolin into the hind paw of 10 cats. $AAR_{2 \text{ mg/kg}}$ (% h) and $AUR_{100 \text{ mg/kg}}$ (% h) are, respectively, the areas above and under the time-response profiles during the 12 h following the administration of 2 and 100 mg/kg robenacoxib. $AAR_{2 \text{ mg/kg}}$ represents approximately 46% of $AUR_{100 \text{ mg/kg}}$ (obtained by subtracting $AUR_{100 \text{ mg/kg}}$ from $AUR_{0 \text{ mg/kg}}$), which indicates that a single SC administration of 2 mg/kg robenacoxib should be associated with a drug response that corresponds to 46% of the maximal drug response that can be achieved with a SC administration of robenacoxib.

the dosing interval equal to EC_{50} (mg/L), Cl (L/kg/day) is the total clearance of robenacoxib and F is bioavailability. Clearance was not determined directly in this study, but Cl/F was estimated using Eqn 2. In contrast to the ED_{50} , the EC_{50} derived from this equation is a genuine PD parameter for the drug and corresponds to the average concentration producing half of the maximum average drug response.

Statistical analysis

Results are presented as mean data and standard deviation (SD). For half-lives, the harmonic mean and corresponding 95% confidence intervals were calculated. For each endpoint, statistical comparisons of responses obtained for different measurement times after robenacoxib administration with pretreatment response were evaluated using a two-way ANOVA (cat factor and measurement time factor). When the time factor was significant, a Dunnett test for multiple comparisons to the pretreatment value was performed (Systat Software Inc., Richmond, CA, USA). A P -value less than 0.05 was considered statistically significant.

RESULTS

Pharmacokinetics

The biexponential decline of robenacoxib blood concentration after SC administration at a dose rate of 2 mg/kg was interpreted as a two-compartment open model with first order absorption (Fig. 3). The apparent total blood clearance (Cl/F i.e., clearance scaled by bioavailability) was relatively high ($10.10 \pm 1.85 \text{ mL/kg/min}$) and the apparent steady-state volume of distribution (V_{ss}/F , volume of distribution scaled by bioavail-

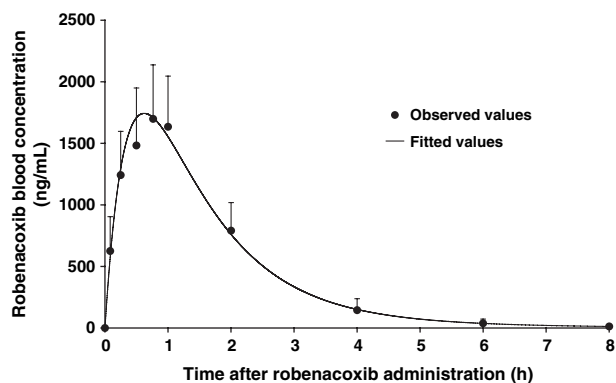


Fig. 3. Observed (mean + SD) and fitted robenacoxib blood concentration (ng/mL) vs. time (h) profile after SC administration at a nominal dose of 2 mg/kg in 10 cats.

ability) was $698 \pm 128 \text{ mL/kg}$. Peak robenacoxib blood concentration ($C_{max} = 1736 \pm 409 \text{ ng/mL}$) was achieved rapidly ($T_{max} = 0.88 \pm 0.13 \text{ h}$). The terminal half-life and the apparent half-life of absorption were 1.87 h (1.34; 3.09) and 0.28 h (0.21; 0.39), respectively.

Pharmacodynamics

Figure 4 illustrates the mean time course, after robenacoxib administration, of six endpoints selected for subsequent PK/PD analysis. For skin and body temperatures and climbing, lameness, pain and descending scores, consistent drug responses were obtained after robenacoxib administration. The mean time of peak response was similar for all endpoints, occurring between 2.6 and 3.5 h after drug dosing (Table 1). The average creeping score also showed good responsiveness to robenacoxib administration. In most animals, the creeping score was highly variable between time points and acceptable fittings could be obtained for a small number of animals only. This endpoint was therefore not considered for further calculations.

Mean minimum body temperature after robenacoxib administration ($38.25 \text{ }^\circ\text{C}$) was similar to mean baseline temperature ($38.56 \text{ }^\circ\text{C}$) before kaolin injection, indicating that complete suppression of hyperthermia was achieved with this dose of robenacoxib. Climbing, descending and pain scores also returned to baseline levels after robenacoxib administration. For skin temperature difference and lameness score, on the other hand, the maximum effect (I_{max}) that could be obtained with high concentrations of the test drug, was consistently smaller than 100% (Table 1), indicating that total suppression of all components of inflammation was not achieved after robenacoxib administration.

Because the onset of the responses was very rapid, the total duration of responses was considered to correspond to the time between drug administration and disappearance of drug responses. Statistical analyses comparing values of each endpoint after drug administration with pretreatment values (obtained just before robenacoxib administration) demonstrated that duration of drug response ranged from 4.6 h (descending

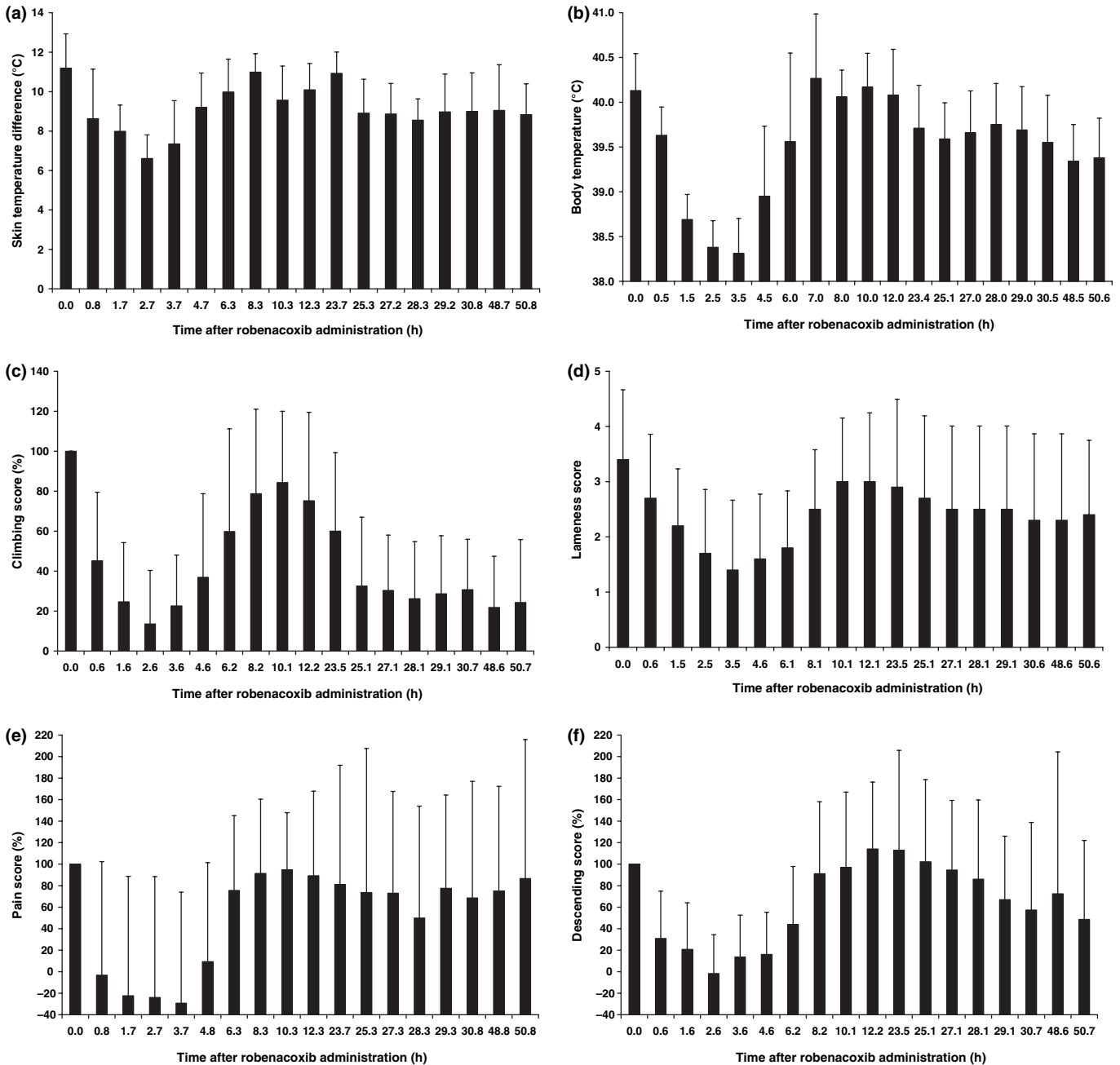


Fig. 4. (a–f) Time course of observed values (mean + SD) in 10 cats for six endpoints [skin temperature difference (a), body temperature (b), climbing score (c), lameness score (d), pain score (e) and descending score (f)] after the administration of 2 mg/kg robenacoxib performed 47 h after injection of 500 mg kaolin into the hind paw.

time) to 8.1 h (lameness score), with an average of approximately 6 h.

As noted for other pharmacodynamic parameters monitored in this study (Giraudel *et al.*, 2005a,b), repeatability (variability of several measurements made at the same time point) was only moderate for withdrawal time measurements [coefficient of variation (CV) of 50.4%]. Nevertheless, when average values for each time point were considered, CVs for the six baseline measurements obtained before inflammation induction ranged from 7.7 to 23.3%, which was considered to be acceptable in view of the marked decrease in pain score obtained after NSAID

administration (average decrease of 163% compared to pretreatment values). Withdrawal times of the control paw were not considered for computation of the pain score, because they seemed to be strongly influenced by the pain in the contralateral paw: the greater the pain in the inflamed paw, the longer was the withdrawal time of the noninflamed paw.

PK/PD modelling

Figure 5a shows for body temperature the onset and offset of responses and the delayed nadir relative to robenacoxib blood

Table 1. Observed and estimated pharmacodynamic parameters (mean and SD) describing robenacoxib anti-inflammatory (skin temperature and lameness, descending and climbing scores), analgesic (pain score) and antipyretic (body temperature) effects after a single SC administration of a nominal dose of 2 mg/kg robenacoxib in 10 cats

Endpoint	T_{\min} (h)	R_{\min} (nu, °C or %)	K_{in} (°C/h or %/h)	R_0 (°C)	R_{\max} (°C)	K_{out} (h^{-1})	I_{\max} (%)	IC_{50} or SC_{50} (ng/mL)	n (no unit)
Body temperature (°C)	2.9 (0.7)	1.88 (0.66)	N.A.	40.4 (0.4)	38.2 (0.5)	1.21 (0.51)	N.A.	105.1 (50.0)	8.3 (2.8)
Lameness score (no unit)	2.6 (0.9)	2.1 (0.7)	4.1 (3.8)	N.A.	N.A.	1.13 (0.98)	70.0 (27.2)	39.2 (25.8)	6.3 (3.8)
Descending score (%)	3.3 (1.6)	109.6 (31.8)	451.5 (548.7)	N.A.	N.A.	3.40 (3.27)	114.2 (41.6)	64.7 (55.4)	4.9 (4.0)
Climbing score (%)	3.5 (1.4)	93.3 (17.0)	185.8 (201.2)	N.A.	N.A.	1.80 (1.96)	94.7 (7.9)	60.7 (41.0)	7.4 (4.2)
Skin temperature difference (°C)	3.1 (0.7)	4.9 (2.4)	14.3 (9.3)	N.A.	N.A.	1.22 (0.89)	57.8 (23.6)	167.8 (113.8)	5.8 (4.5)
Pain score (%)	2.7 (1.3)	163.1 (124.3)	112.0 (64.4)	N.A.	N.A.	0.99 (0.55)	118.1 (64.4)	111.5 (54.5)	7.8 (3.5)

T_{\min} and R_{\min} [same unit as endpoint, i.e. nu (no unit) for the lameness score, % or °C] are observed values for the time of occurrence of the peak response and the maximum robenacoxib response expressed as a decrease in the endpoint value, respectively. Data were fitted with an indirect response model in which robenacoxib produces its pharmacodynamic effect by inhibiting the factors controlling K_{in} . The rate of change of the response over time and the signification of K_{in} , K_{out} , IC_{50} , EC_{50} , I_{\max} and n (upper bound fixed at 10) are given by Eqn 4. For body temperature another indirect response model was used describing drug effect as stimulating heat loss (Eqn 6). N.A., not applicable in the model.

concentrations. Simultaneous PK/PD modelling was undertaken to deal with this temporal delay between PK and PD data, which was also present for the other endpoints selected (Fig. 5b, c). A number of models were tested for the drug effect: the sigmoid E_{\max} model gave a significantly better description of the response vs. time data compared with the E_{\max} model (Hill coefficient set to 1). Robenacoxib sensitivity (i.e. the slope of the concentration-effect relationship) was relatively high for all the endpoints, ranging from 4.9 to 8.3 (Table 1) and illustrates that there was an almost 'all or nothing' effect on the input and output processes of the indirect response models. The relatively high inter-animal variability observed for most endpoints translated into a high variability for the model and drug parameters [e.g. CVs for potency ranged from 48% (body temperature) to 86% (descending score)]. For most parameters, there were also differences depending on the endpoint considered. This was especially true for potency (IC_{50} or SC_{50}), with mean values ranging from 39 ng/mL (lameness score) to 168 ng/mL (skin temperature difference) (Table 1).

Data sets for which acceptable PD fittings were obtained and for which drug and model parameters were estimated with adequate precision, were used to simulate average drug response profiles for several dosage regimens. Figure 6 illustrates for three endpoints the simulated average drug response profiles for doses of robenacoxib ranging from 0.1 to 10 mg/kg. It is predicted that the lowest dose (0.1 mg/kg) would produce a very small drug response, 0.5 mg/kg would provide nearly maximal efficacy but drug response would be transient, whilst a 2 mg/kg dose would provide good efficacy during 5 to 7 h. Several dosage intervals were also simulated (up to 10 000 mg/kg to simulate maximum possible drug response over 24 h) and the model predicts better analgesic and anti-inflammatory efficacy for 1 mg/kg robenacoxib given every 12 h than for 2 mg/kg given once a day (Fig. 7). Average drug response is increased by 61–69% when

the same total daily dose is divided into two equal doses at 12 h intervals.

Table 2 presents simulated average drug responses for several dosage regimens (single dosage of 0.5 to 10 mg/kg robenacoxib) and the doses producing 40%, 50% and 60% of the maximum possible average drug response that can be obtained with a single SC administration of robenacoxib. For each endpoint the ED_{50} was then used to compute an EC_{50} representing the average robenacoxib blood concentration producing half of the maximum drug response after a single SC administration (Table 3).

DISCUSSION

Knowledge of concentration-effect relationships can provide valuable insights into mechanisms of drug action (Landoni *et al.*, 1995b; Torres-Lopez *et al.*, 1997; Flores-Murrieta *et al.*, 1998; Josa *et al.*, 2001; Lees *et al.*, 2004) and may also be used to optimize dosage regimens (Toutain *et al.*, 2001; Toutain, 2002; Giraudel *et al.*, 2005a,b). This study focused on the application of PK/PD modelling to the selection of an effective dosage regimen for subsequent clinical evaluation for a new NSAID. For this purpose, clinically relevant endpoints such as lameness were recorded. Other endpoints, including skin temperature and pain score investigated more specific components of the inflammatory response (local heat production and hyperalgesia) and thus provided information with a more mechanistic interest.

The time required for the cats to perform each of the three locomotion tests was analysed separately and calculation of descending and climbing scores led to similar time profiles to that obtained for lameness scoring. This finding supported the hypothesis that these three measurements can also be considered

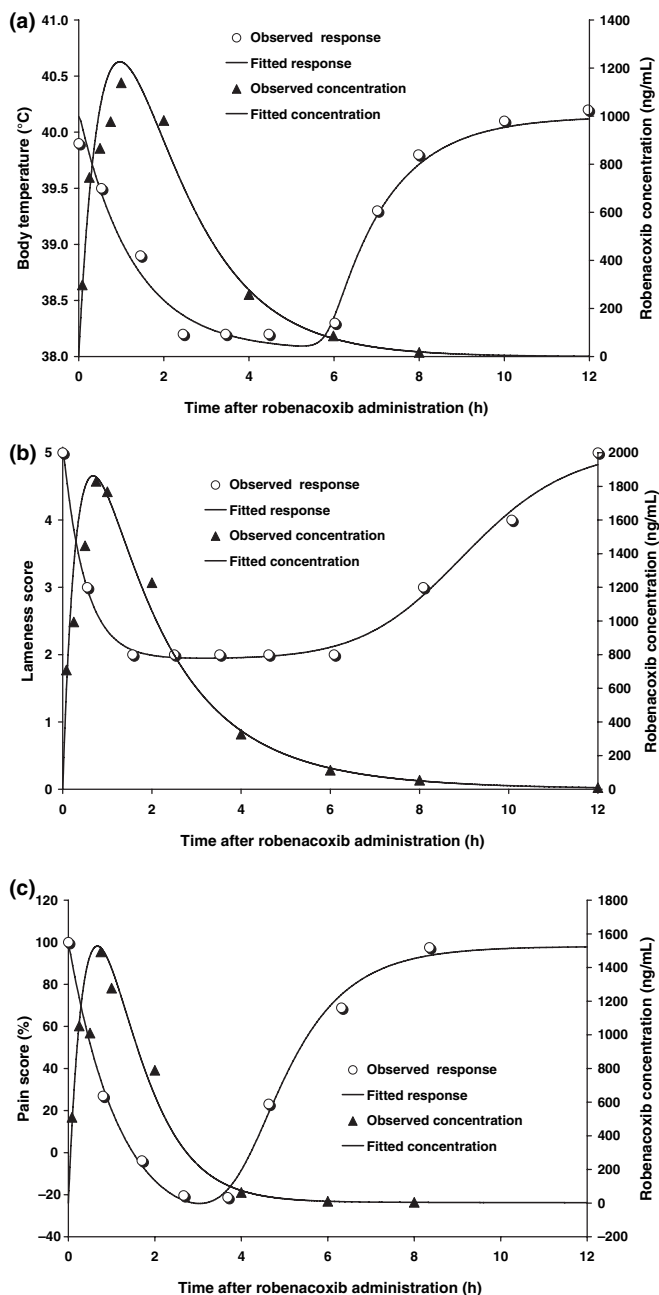


Fig. 5. (a–c) Time courses in single representative cats of observed and fitted robenacoxib blood concentration (ng/mL) and body temperature (a), lameness score (b) and pain score (c).

as clinically relevant surrogate endpoints. Lameness and locomotion scores probably reflect both the pain experienced by the cat and functional impairment because of oedema, and therefore do not allow discrimination between pain relief and other anti-inflammatory effects. Nevertheless, because they may be more clinically relevant than other endpoints, they were important from the perspective of establishing an effective clinical dosage regimen for robenacoxib. Skin temperature rise, on the other hand, is not a direct clinical endpoint, but it is a marker of the anti-inflammatory effect of NSAIDs (Giraudel *et al.*, 2005a,b).

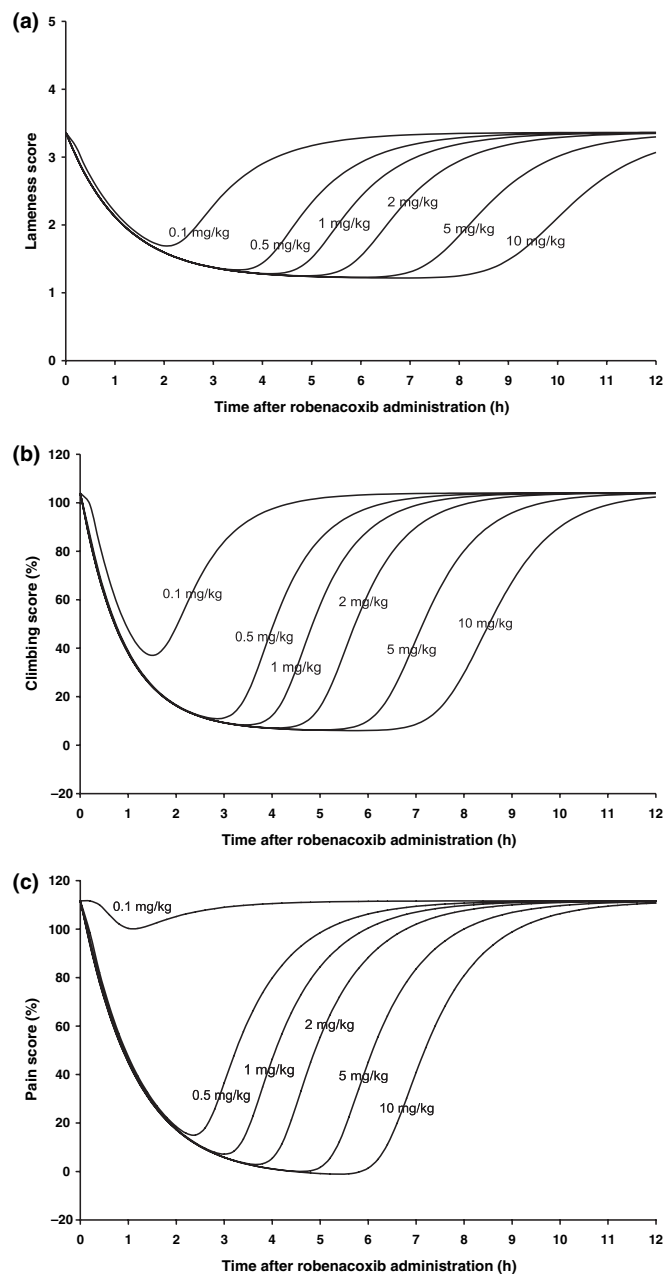


Fig. 6. (a–c) Simulated time profiles of lameness score (a), climbing score (b) and pain score (c) for six single subcutaneous dose administrations of 0.1, 0.5, 1, 2, 5 and 10 mg/kg robenacoxib.

Similarly, the time required for paw withdrawal, in response to the radiant heat emitted by an analgesia meter, cannot be considered to reflect solely the kaolin-induced pain experienced by the cat. The fact that normal cats had a withdrawal response of the same nature (although reaction was obtained after a longer time) suggests that the response to the heat stimulus can be termed hyperalgesia (enhanced response to a noxious stimulus). However, this does not exclude the existence of allodynia (nocifensive behaviour in response to an innocuous stimulus normally not perceived as painful) or other types of pain in this experimental inflammation.

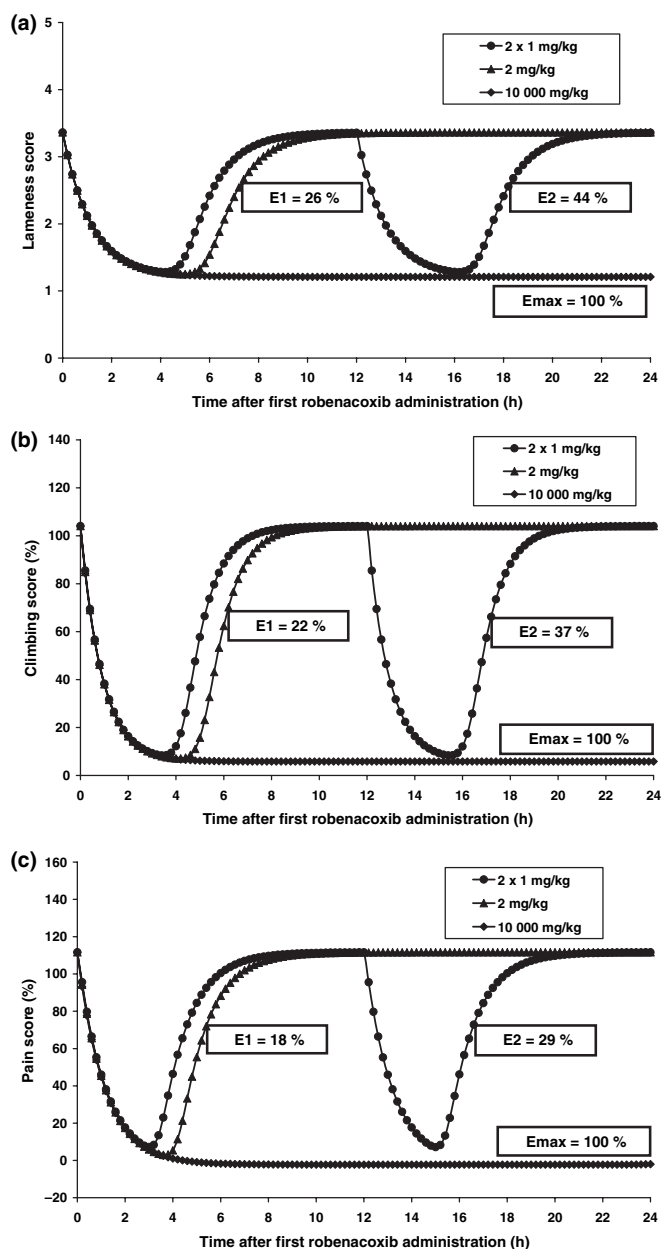


Fig. 7. (a–c) Simulated time profiles of lameness score (a), climbing score (b) and pain score (c) for two dosage regimens: a single SC dose of 2 mg/kg robenacoxib and two SC doses of 1 mg/kg, the second dose being administered 12 h after the first injection. E1 (2 mg/kg) and E2 (2 × 1 mg/kg) represent the average drug response expressed as a percentage of the maximum possible average drug response (E_{max}) i.e., the theoretical response obtainable for an infinite single dose of robenacoxib (e.g., 10 000 mg/kg).

Achieving objective and quantitative measurement of experimental pain and then establishing the extent and time course of analgesia presents many difficulties. Analgesic visual analogue scales and pain scoring systems have been used as subjective indices to compare the clinical manifestations of pain in cats receiving NSAIDs pre- or postoperatively with those of animals given saline or drugs of other classes (Lascelles *et al.*, 1995;

Slingsby & Waterman-Pearson, 1998, 2002). Mechanical nociceptive thresholds have been used by some authors (Slingsby & Waterman-Pearson, 2000; Slingsby *et al.*, 2001) to provide more objective and quantitative measurements of pain. In our hands, pain elicited by digital pressure or with a pressure algometer (electronic von Frey transducer) resulted in high inter- and intra-animal variability and strong habituation (withdrawal of the paw as soon as the probe was applied against the skin, even in the absence of inflammation) (Giraudel *et al.*, 2005b). Thermal threshold testing has shown better sensitivity than mechanical stimulation (Hargreaves *et al.*, 1988) and has previously been used in cats (Booth & Rankin, 1954; Dixon *et al.*, 2002). In this study, the pain score determined by thermal sensory testing provided an appropriate measure of hyperalgesia.

All endpoints used in this study showed good responsiveness to robenacoxib and both magnitude and time courses of effect were well described by indirect response models. In contrast to body temperature and climbing, descending and pain scores, complete suppression of lameness and skin temperature increase were not achieved in most animals, with a maximal effect (I_{max}) for the latter endpoints equal to 70% and 58%, respectively. A similar finding was reported for meloxicam in a previous feline study (Giraudel *et al.*, 2005a), which may indicate that total suppression of the inflammatory response may not be possible with NSAIDs in this challenging model of inflammation. For locomotion scores (climbing and descending tests), on the other hand, the theoretical maximum efficacy (100%) was achieved. In consequence, it can be concluded that the information provided by these two locomotion tests differs from that obtained with the numerical rating scale (NRS) for lameness, further demonstrating the usefulness and discriminative value of these two quantitative endpoints.

As well as magnitude, the second clinically relevant variable is the duration of drug response. The kaolin-induced inflammation was sufficiently protracted to establish this duration, which was found to be similar for all endpoints (approximately 6 h). Nevertheless, predictions of *in vivo* efficacy based on inflammation models have limitations. Most important is the clinical relevance of the model itself. Kaolin induces a relatively challenging inflammatory response, associated with local persistence of the foreign body at the inflammation site, whereas in many clinical situations inflammation may be less intense or the underlying stimulus less persistent. Furthermore, this inflammation model does not and is not intended to mimic inflammatory joint diseases, for which accumulation in and slow clearance from the synovial compartment have been demonstrated for several NSAIDs (Brune & Furst, 2007; Armstrong *et al.*, 1999; Scott *et al.*, 2004). Extravascular accumulation of NSAIDs has also been extensively studied in polyester sponge and tissue cage models of carrageenan-induced inflammation, with measurement of persistence of drug concentrations in inflammatory exudate (Landoni *et al.*, 1995a, 1999; Lees *et al.*, 1999, 2004).

In human rheumatoid arthritis patients treated with the COX-2 selective drug lumiracoxib, the mean steady-state trough concentration in synovial fluid was approximately three times

Table 2. Simulated robenacoxib average drug responses (expressed as percentages of the maximum possible average drug response, i.e. the response obtained with 100 mg/kg robenacoxib) and 40%, 50% and 60% effective doses for different endpoints

Endpoint	Average response (%)					Doses (mg/kg)		
	E _{0.5} *	E ₁ *	E ₂ *	E ₅ *	E ₁₀ *	ED ₄₀ †	ED ₅₀ †	ED ₆₀ †
Body temperature	24.1	31.2	38.4	49.1	58.8	2.3	5.4	10.8
Lameness score	37.9	45.9	55.0	70.3	84.5	0.6	1.4	2.8
Descending score	29.0	36.1	43.7	55.9	67.5	1.4	3.3	6.5
Climbing score	31.0	38.3	46.3	58.9	71.2	1.2	2.7	5.4
Skin temperature	19.2	26.8	34.0	44.3	53.2	3.5	7.9	15.7
Pain score	23.7	31.0	38.4	49.2	59.0	2.3	5.3	10.6

*E_X (%) represents the predicted average drug response for a single subcutaneous dose of X mg/kg robenacoxib.

†ED_Y (mg/kg) is the robenacoxib dose producing Y % of the maximum possible average drug response that can be obtained with a single SC administration of robenacoxib.

Table 3. Predicted COX-1 and COX-2 inhibition levels corresponding to different *in vivo* determined target concentrations

Endpoint	Robenacoxib pharmacological effect			Robenacoxib clinical response		
	IC ₅₀ or SC ₅₀ * (ng/mL)	COX-1 inhibition† (%)	COX-2 inhibition† (%)	EC ₅₀ ‡ (ng/mL)	COX-1 inhibition† (%)	COX-2 inhibition† (%)
Body temperature	105.1	2.3	80.0	371.4	6.3	91.7
Lameness score	39.2	1.0	64.4	96.3	2.2	78.9
Descending score	64.7	1.6	73.0	227.0	4.3	88.2
Climbing score	60.7	1.5	72.0	185.7	3.7	86.4
Skin temperature	167.8	3.4	85.4	543.4	8.5	93.8
Pain score	111.5	2.4	80.8	364.6	6.2	91.6

*Average potencies obtained with indirect response models for the different endpoints.

†Levels of COX inhibition are predicted percentages corresponding to different *in vivo* determined concentrations (IC₅₀, SC₅₀ or EC₅₀) and computed with results obtained in feline whole blood assays (Giraudel *et al.*, 2008).

‡Predicted average concentration for a dose of robenacoxib equal to the ED₅₀ for each endpoint (see Materials and Methods).

higher than the mean plasma concentration (Scott *et al.*, 2004). This prolonged persistence in the synovial compartment was also demonstrated for diclofenac (Fowler *et al.*, 1986). The high affinity with which NSAIDs bind to albumin and the subsequent degradation of this protein by lysosomal enzymes (McCormack & Brune, 1991) may explain the high drug concentrations achieved in inflamed tissues (Fowler *et al.*, 1986; Weaver *et al.*, 2003; Scott *et al.*, 2004). Unlike diclofenac, lumiracoxib has a lipophilic character, which facilitates rapid distribution to sites of inflammation and probably accounts for the rapid onset of its analgesic effects (Zelenakas *et al.*, 2004). The chemical structure of robenacoxib closely resembles that of diclofenac and lumiracoxib. Moreover, it is both a weak acid and lipophilic, which may explain the rapid onset of response observed in this study. If robenacoxib persists longer in the synovial space than in blood because of its physico-chemical characteristics, as demonstrated for diclofenac and lumiracoxib, it may have prolonged local effects when blood concentrations decrease below effective levels. Indeed, robenacoxib has been shown to have a long residence time in zymosan and carrageenan induced inflammatory exudate in tissue cages in rats and cats (King *et al.*, 2008; Pelligand *et al.*, unpublished data).

Unlike efficacy and duration of drug response, potency and sensitivity (slope of the concentration-effect relationship) are

parameters of indirect rather than direct clinical interest. However, they are crucial for selection of an effective dosage regimen. Sensitivity provides information on the range of efficacious concentrations or doses. A consequence of the high values for sensitivity for all endpoints obtained in this study is that there is probably a threshold concentration below which no drug effect occurs. Furthermore, because potencies determined *in vivo* in this study are assumed to reflect robenacoxib inhibition of prostaglandin synthesis, it is relevant to compare these IC₅₀s with the potency for COX-2 inhibition by robenacoxib previously reported in feline whole blood assays (Giraudel *et al.*, 2008). These *in vitro* potencies were compared with potencies obtained after PK/PD modelling (potencies for drug effect on production of inflammatory mediators) and potencies computed after simulations (potencies for drug response i.e., decrease of the values of each endpoint) (Table 4). These comparisons were performed by calculating, for the potencies determined *in vivo*, the corresponding COX inhibition levels that would be achieved with these concentrations (Table 4). It is clear that potencies for drug effect and potencies for drug response give different predictions, but, for all concentrations of robenacoxib, it is predicted that COX-1 inhibition would not exceed 8.5%, whereas COX-2 would be inhibited by at least 64%. It is therefore likely that the COX-2 selectivity demonstrated *in vitro* (Giraudel *et al.*,

Table 4. Predicted inhibition percentages of COX-1 and COX-2 for different single dose administrations of robenacoxib. Blood concentration vs. time profiles were simulated with average PK parameters obtained after SC administration of 10 cats with 2 mg/kg robenacoxib. ICs (concentrations producing different levels of COX-1 and COX-2 inhibition) were obtained in feline whole blood assays (Giraudel *et al.*, 2008)

Parameter	Dose (mg/kg)		
	1	2	5
% inhibition of COX-1 corresponding to the average blood concentration*	2.9	5.1	10.3
% inhibition of COX-2 corresponding to the average blood concentration	83.4	89.8	94.9
% inhibition of COX-1 corresponding to the maximal blood concentration	12.0	19.6	34.3
% inhibition of COX-2 corresponding to the maximal blood concentration	95.7	97.5	98.8
Time above IC ₅₀ for COX-2 (h) [†]	5.4	6.5	8.4
Time above IC ₈₀ for COX-2 (h)	3.3	4.1	5.3
Time above IC ₁₀ for COX-1 (h)	1.0	2.0	3.0
Time above IC ₂₀ for COX-1 (h)	0.0	0.0	1.9

*Predicted level of COX-1 inhibition (%) corresponding to the average robenacoxib concentration achieved over the first 12 h following a single SC administration of robenacoxib.

[†]Predicted time (h) during which the robenacoxib blood concentrations exceed a concentration equal to the IC₅₀ for COX-2, IC₅₀ and IC₈₀ for COX-2 are 18.9 and 104.9 ng/mL, respectively. IC₁₀ and IC₂₀ for COX-1 are 674.8 and 1788.8 ng/mL, respectively.

2008) for robenacoxib holds true for clinically relevant concentrations *in vivo*.

The pharmacokinetic profile of robenacoxib confirms previously published data (total clearance, 0.63 L/kg/h; bioavailability, 94%) (Giraudel *et al.*, 2008). As the dosage regimen used in this study provided good clinical efficacy, it was relevant to compare these clinically effective blood concentrations with concentrations obtained in feline whole blood assays of COX-1 and COX-2 inhibition (Giraudel *et al.*, 2008). This comparison was performed by simulating blood concentration profiles for three dose levels (1, 2 and 5 mg/kg robenacoxib), and the corresponding predicted COX-1 and COX-2 inhibition levels were calculated (Table 4). The results indicate a high and consistent degree of COX-2 inhibition (83 to 95%), which corresponds to what has been commonly reported for NSAIDs in humans (Warner *et al.*, 1999). COX-1 is only marginally inhibited, except for the 5 mg/kg dose, for which COX-1 inhibition could exceed 20%, but only for 2 h (Table 4). Therefore, robenacoxib is one of the first NSAIDs developed for veterinary medicine for which only minimal COX-1 inhibition is obtained at clinically effective doses in the cat. This might explain the good tolerance of robenacoxib at doses 5 to 10 times higher (i.e. 10 to 20 mg/kg, Novartis, unpublished data) than the doses anticipated for clinical use. Furthermore, if administered once daily, another advantage of robenacoxib in terms of safety could be its short blood half-life. There is increasing evidence that COX-2 activity is related to the synthesis of anti-inflammatory prostanoids in the resolution phase of the acute inflammatory process (Gilroy *et al.*, 1999; Willoughby *et al.*, 2000). As this phase is associated with a secondary increase in COX-2 expression and is not always easy to recognize clinically, it may be useful to avoid abolition of COX-2 activity over the whole dosing interval.

When using indirect response modelling, the final simulation step giving EC_{50s} and ED_{50s} (or indeed any ECs and EDs) for drug response may be used to predict clinically effective dosage regimens. ED_{50s} for clinically relevant endpoints are classically used for this purpose (Toutain *et al.*, 2001). In this study, ED_{50s} for the lameness and locomotion scores were determined,

indicating an estimated clinically effective dose of 1.5 to 3.5 mg/kg for SC administration of robenacoxib in the cat. The fact that doses computed for body temperature were similar or higher than those obtained for endpoints reflecting anti-inflammatory effects contrasts with what is classically observed for NSAIDs (Toutain *et al.*, 2001; Lees, 2003). Antipyretic doses required to achieve 50% of the maximal drug response (ED_{50s} for body temperature) are usually lower than corresponding anti-inflammatory doses. The present finding is, however, in accord with previous studies in the rat showing that robenacoxib has a lower potency for suppression of hyperthermia compared with its analgesic and anti-inflammatory effects (King *et al.*, 2008).

Another conclusion from the simulations undertaken in this study was that drug effectiveness for robenacoxib (i.e., the effect per unit of drug concentration) was dependent on drug delivery rate and that twice a day administration schedules might give better overall drug responses than once daily dosing. However, the analogue of robenacoxib, lumiracoxib, actually provided a slightly better efficacy with once daily dosing of 400 mg compared with 200 mg twice daily in humans with osteoarthritis, and this finding may be explained by the relatively long residence time of lumiracoxib at sites of inflammation (Scott *et al.*, 2004; Berenbaum *et al.*, 2005). The same may be true for robenacoxib, therefore the efficacy of once vs. twice daily dosing with robenacoxib requires evaluation in clinical studies.

In conclusion, robenacoxib was shown to exert good analgesic and anti-inflammatory efficacy in a kaolin-induced model of inflammation and pain in the cats. This study further demonstrated that the potency of robenacoxib as an inhibitor of COX-2 (previously demonstrated) was of the same order of magnitude as the potencies estimated with the present PK/PD models. The selected indirect response models allowed for a good temporal resolution of robenacoxib effects and permitted determination of clinically effective concentrations, for which COX-1 inhibition is predicted to be very low. The PK/PD models were additionally used for simulating several dosage regimen scenarios that will assist the planning of dosages for evaluation in clinical trials.

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