Paw Inflammation Model in Dogs for Preclinical Pharmacokinetic/Pharmacodynamic Investigations of Nonsteroidal Anti-Inflammatory Drugs


Received December 17, 2010; accepted April 26, 2011

ABSTRACT

The goal of the present study was to develop and validate a new canine model of inflammation. The motivation was to make available a scientifically appropriate and ethically acceptable model to conduct pharmacokinetic/pharmacodynamic investigations for testing nonsteroidal anti-inflammatory drugs in dogs. A kaolin-induce paw inflammation model previously developed in cats was adapted to the dog. The paw inflammation developed within a few hours, reached maximum values 24 h and up to 3 days after kaolin administration, and then progressively resolved over 2 months. Five end points of clinical interest (body temperature, creeping time under a tunnel, paw withdrawal latency to a standardized thermal stimulus, lameness score, and vertical force developed during walking on a force plate) were measured regularly over the next 24 h and beyond to characterize the time development of the inflammation either in control conditions (placebo period) or after the administration of meloxicam (test period) according to a crossover design. Pharmacodynamic data were modeled using an indirect response pharmacokinetic/pharmacodynamic model. This model described three effects of meloxicam, namely, classic anti-inflammatory, analgesic, and antipyretic effects. The mean plasma meloxicam IC50 values were 210 ng/ml for the antipyretic effect, 390 ng/ml for the analgesic effect, and 546 ng/ml for the vertical force exerted by the paw on the ground as measured by force plates. These in vivo IC50 values require approximately 80 (antipyretic effect) to 90% (all other effects) cyclooxygenase-2 inhibition as calculated ex vivo whole-blood assay data.

Introduction

Few preclinical studies have attempted to model plasma concentration-time profiles with the time course of nonsteroidal anti-inflammatory drug (NSAID) effects in small carnivores (Toutain et al., 2001; Giraudel et al., 2009). A requisite to carry out a pharmacokinetic/pharmacodynamic (PK/PD) dose determination is the availability of an animal model in which the measured end points are clinically relevant, measurable with good metrological performances, and long enough to prevent (or minimize) confusion between the action of the investigated NSAID and the natural time development of the inflammatory process. Currently, only acute knee joint synovitis using intra-articular injection of sodium urate crystals has been well described in dogs, and its application to the preclinical evaluation of different NSAIDs is now well documented (Cross et al., 1997). However, this model is of too short in duration (less than 24 h) to allow a PK/PD investigation for drugs having a duration of action of approximately 24 h. Likewise, the carrageenan-induced acute paw inflammation in dogs model proposed by Brooks et al. (1991) was too short in duration for this purpose. This is why we developed a Freund’s complete adjuvant osteoarthritis model, resulting in a sustained and relatively stable secondary inflammatory response (Botrel et al., 1994; Toutain et al., 2001). This Freund’s model was scientifically attractive, allowing repeatable measurements of relevant clinical end points, but the inflammation was irreversible, and it was mandatory to sacrifice dogs for ethical reasons. Because we are committed to a program of rehabilitating dogs by donating them as companion animals at the end of the trials, we adopted the 3R principles to avoid unnecessary suffering to
animals [i.e., replace (use alternatives to animal testing whenever possible), reduce (improve existing methods so that fewer laboratory animals are required in an experiment), and refine (improve existing methods so that animals experience as little discomfort and distress as possible)]. The PK/PD approach itself can be considered as a refinement of a dose titration allowing a reduction in the required number of animals to establish a full dosage regimen (dose and dosing interval), but to achieve our ultimate goal of rehabilitating experimental animals, it was necessary to develop and validate a new reversible inflammation model in dogs. Such a model was developed recently in cats (Giraudel et al., 2005a). It consisted of administering kaolin (an inert foreign body) as a phlogistic agent in the paw. The resulting inflammation was sustained between 1 and 3 days after kaolin injection, allowing administration of the NSAID on day 2. The model was found to be suitable for simultaneously studying the analgesic, anti-inflammatory, and antipyretic effects of NSAIDs in cats by measuring different end points such as body temperature or gait scoring (Giraudel et al., 2005a,b).

Moreover, because the kaolin was administered extra-artistically, the inflammation progressively vanished with either the physical wasting of the administered kaolin by direct skin exudation or the encystment of the remnant kaolin fraction, and within a few weeks, most animals returned to the control condition without any apparent sequelae.

The objective of the present study was to transfer and validate this reversible inflammation model in dogs, a species used extensively in regulatory studies in both human and veterinary medicine. We selected meloxicam as a test NSAID, because it is a well established NSAID in dogs (Aragon et al., 2007) and also used in man.

**Materials and Methods**

**Animals.** Eight healthy Beagle dogs (two females and six males) were selected after clinical examination and biochemical analysis. The body weights and ages of the dogs ranged from 12.5 to 17.5 kg and 1 to 3 years, respectively. Between experimental phases, the dogs were housed in large boxes. During the different phases of the trials, the animals were kept in individual stainless steel cages in a controlled environment. On the days of the measurements, the dogs were placed in their cages at least 2 h before any measurement. Dogs were fed each evening after the last measurements with 250 ± 50 g of commercial dry food (medium; Royal Canin, Aimargues, France). Animal care and conduct of the study were performed according to the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, 1996). The study has been carried out in accordance with the Declaration of Helsinki and with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the U.S. National Institutes of Health. The protocol was approved by the Animal Experimentation Ethics Committee of Midi-Pyrenees. The study was performed in compliance with the Principles of Good Clinical Practice (CVMP/VICH/595/98; http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/10/WC500004343.pdf) and according to the Guideline for the Conduct of Efficacy Studies for NSAIDs (EMEA/CVMP/237/01; http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/10/WC500004243.pdf). Because the model is totally reversible without sequelae, all of the dogs that participated in this trial were rehabilitated as companion animals at the end of the study.

**Animal Preparation and Induction of Inflammation.** Both hind paws were shaved from the toes up to the hock joint, and targets were marked for the pain assessment and paw circumference measures (Fig. 1).

![Fig. 1. Cutaneous reference marks on the hind paw of the dog for the circumference (●) and analgesia (○) measurements and view of the different sites of subcutaneous kaolin injection (→).](image-url)

The preparation of kaolin (hydrated aluminum silicate; Sigma-Aldrich, St. Louis, MO) was done using standardized procedures (Giraudel et al., 2005b). For the induction of inflammation, the dogs were given a general anesthetic with alfaxalone 3 mg/kg (Alfaxan; Vetoquinol, Lure, France), and anesthesia was maintained with iso-flurane (2% v/v; Aerrane; Baxter, Lessines, Belgium). A local anesthetic was added: approximately 2.5 ml of lidocaine was injected subcutaneously into the paw (Lurocaine; Vetoquinol), after which 1.85 ± 0.10 g of kaolin was injected under aseptic conditions subcutaneously at eight different points in the metatarsal pad. The total volume of the kaolin suspension (25% w/v) was 6.4 ml.

**Experimental Design.** The eight dogs and the investigators were trained regularly for all of the experimental conditions and all of the end point measurements for at least 1 month. This training period guaranteed the eligibility for the trial of the eight selected dogs and the investigators’ skills. The trial consisted of two phases. In the first phase, the morphological performances of the different end points were assessed in all of the dogs. Investigated end points were body temperature, paw circumference, time to perform a creeping test under a tunnel, vertical force of the paw on force plates, and thermal pain threshold. Three series of end point measurements were obtained each day and for 5 consecutive days, and the repeatability and reproducibility of the different end points were computed. Lameness scoring was not validated during this first control phase because all of these control measurements were obtained in non-inflammatory conditions. Then, during a second phase, a two-period, two-sequence crossover design was carried out. The eight dogs were allocated randomly into four pairs of the same sex and of similar body weight. Dogs of each pair then were allocated into one of two groups (sequences) corresponding to the order of the administration of the test articles (placebo then NSAID or, conversely, NSAID then placebo). The washout period between the two periods of the crossover was 8 weeks. Kaolin inflammation was induced for each dog of a pair and in the two periods (right paw period 1 and left paw period 2). The experiment was blinded to the investigators. The placebo corresponded to a sham meloxicam administration. Meloxicam (Metacam; Boehringer Ingelheim Vetmedica, Ingelheim/Rhein, Germany) was administered by the subcutaneous route 26 h after kaolin inflammation induction. The dose of meloxicam administered was the marketed dose for dogs (0.2 mg/kg). Blood samples were obtained from the jugular vein by direct puncture at time 0 (control) and then 5, 15, 30, and 60 min and, 2, 4, 6, 9, 12, 21, 48, and 72 h after the test article administration (meloxicam or placebo). Before kaolin administration, the PD end points were measured twice the same day and then twice at 22 and 24 h after the induction of the kaolin inflammation. Subsequently, the PD end points were measured at 30 min, 2, 25, 4, 6, 9, 12, 15, 18, 21, 30, and 48 h, and 3, 4, 5, 6, 7, 10, 14, 17, 21, and 24 days after test article administration (i.e., placebo or any other drug).
TABLE 1
Numerical rating scale for the evaluation of lameness in the inflamed paw in dogs

<table>
<thead>
<tr>
<th>Score</th>
<th>Definition of the Lameness Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No lameness</td>
</tr>
<tr>
<td>1</td>
<td>Barely detectable lameness over most of the observation period</td>
</tr>
<tr>
<td>2</td>
<td>Mild lameness, substantial weight bearing</td>
</tr>
<tr>
<td>3</td>
<td>Moderate lameness, minimal weight bearing</td>
</tr>
<tr>
<td>4</td>
<td>Severe lameness, the animal uses his paw (walking movement initiated and/or touches lightly the ground) but does not bear weight</td>
</tr>
<tr>
<td>5</td>
<td>The animal could not be more lame, refuse to move and/or avoid any contact of the inflamed paw with the ground</td>
</tr>
</tbody>
</table>

The method was linear over the calibration range from 10 to 1500 ng/ml. Eluted at retention times of 7.2 and 5.6 min, respectively. The mobile phase used for the analysis consisted of a 50:50 mixture of water with 1% acetic acid and acetonitrile (Sigma-Aldrich) at a flow rate of 0.2 ml/min. Under these conditions, meloxicam was verified on blank plasma from untreated dogs, establishing the specificity of the method. The validated limit of quantification was 10 ng/ml.

Data Analysis. PK and PK/PD modeling were performed by least-squares regression analysis using WinNonlin Professional software (WinNonlin, version 5.2, Pharsight Corporation, Mountain View, CA).

Individual plasma meloxicam concentrations (ng/ml) were fitted to polynoexponential equations. The data points were weighted by the inverse of the squared-fitted value. The number of exponents (two or three) needed to obtain the best fit for each data set was determined by the Akaike’s information criterion (Yamaoka et al., 1978) and by inspection of the plot of residuals. On the basis of this approach, a biexponential equation corresponding to a monocompartmental model for extravascular administration with a lag time was selected (eq. 1).

\[ C(t) = \frac{FDk_{01}}{V(k_{01} - k_{02})} \left( \exp(-k_{01} \times (t - t_{lag})) - \exp(-k_{02} \times (t - t_{lag})) \right) \]

where \( C(t) \) is the meloxicam plasma concentration (ng/ml) at time \( t \) (h), \( V/F \) (l/kg) is the apparent volume of distribution, \( k_{01} \) (h) is the rate constant of the initial ascending phase, \( k_{10} \) (h) is the rate constant of the terminal phase, and \( D \) is the meloxicam dose (mg/kg). The parameters \( (V/F, k_{01}, k_{10} \) and \( t_{lag} \) were estimated.

For PK/PD modeling, raw data were used directly as dependent variables for body temperature, lameness score, and creeping time. Because kaolin decreased the vertical force exerted on the force plate or the paw withdrawal time, these variables were transformed to be expressed as a percentage of reduction (100% is the maximal effect observed after kaolin administration) to use the same PK/PD model for all endpoints. Individual PK/PD relationships were described using indirect PD response models (Dayneka et al., 1993). In these models, the measured response \( R \) is assumed to result from factors controlling either the input or the dissipation of the measured response. Different models were explored, including precursor-dependent indirect response models (Sharma et al., 1998), to capture not only the main effect of meloxicam but also the development of rebound phenomena, which were observed after meloxicam action had ceased. Finally, PD data were described by the following sets of differential equations (eqs. 2–4).

In the control condition (i.e., before kaolin administration), for both periods

\[ \frac{dR}{dt} = k_{in} - k_{out}R \]

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

The time development of the kaolin action, noted as \( kao(t) \), on the different measured responses was incorporated additively to \( k_{in} \) in the model described by eq. 3

\[ \frac{dR}{dt} = k_{in} + kao(t) - k_{out}R \]

where \( kao(t) \) is the zero-order input rate function (same units as \( k_{in} \)) representing the action of the kaolin accounting for the temporal increase in the response after kaolin administration. Such a model already has been selected by other researchers for the PK/PD modeling of the antipyretic effect of naproxen in a model of endotoxin-challenged rats (Josa et al., 2001). After consideration of the inverse U shape of the time development of the responses in the placebo period, \( kao(t) \) was modeled using an empirical biexponential equation of the form (eq. 4)

\[ kao(t) = P1[\exp(-P2(t - t_{lag})) - \exp(-P3(t - t_{lag}))] \]
with $P_1$ the intercept (response unit per hour), $P_2$ (1/h) the slope of the decreasing phase of the kaolin action, $P_3$ (1/h) the rate constant reflecting the increasing phase of the kaolin action after kaolin administration, and $t_{\text{out}}$ the time of kaolin administration. The three parameters ($P_1$, $P_2$, and $P_3$) were estimated simultaneously with the PD parameters of ultimate interest (i.e., $k_{\text{in}}$, $IC_{50A}$, and $k_{\text{out}}$; see below), and the lag time was fixed at the actual time of kaolin administration for both placebo and test periods.

The effect of meloxicam (melox) was described as the consequence of the inhibition of the phlogogenic effect of kaolin, that is, $kaol(t)$ and was incorporated in eq. 3 as (eq. 5)

$$\text{melox} = \frac{I_{\text{max}} \times C(t)^{n1}}{IC_{50A} + C(t)^{n2}}$$

(6)

where $C(t)$ is the meloxicam plasma concentration at time $t$ (ng/ml) is the meloxicam plasma concentration producing half the maximum meloxicam effect (i.e., $50\%$ of $I_{\text{max}}$), $I_{\text{max}}$ is the meloxicam plasma concentration producing half the maximum meloxicam effect (i.e., $50\%$ of $I_{\text{max}}$), $IC_{50A}$ is the meloxicam plasma concentration producing half the maximum meloxicam effect (i.e., $50\%$ of $I_{\text{max}}$), $n1$ is the slope of the concentration-effect relationship. For the test period, it was nearly always observed that after the meloxicam action vanished the response increased again reflecting the increasing phase of the kaolin action after kaolin administration halving the plasma meloxicam concentration 

$$\text{delaying the disappearance of the kaolin action, and the natural time measurement}$$

and $t_{\text{out}}$ was fixed at the actual time of kaolin administration for both placebo and test periods.

To estimate the extent of cyclooxygenase (COX)-1 and COX-2 enzyme inhibition corresponding to the different estimated in vivo $IC_{50A}$ values, we computed the percentage of COX-1 and COX-2 inhibition from ex vivo results of a whole-blood assay performed in another group of Beagle dogs. COX-1 activity was determined by measuring serum thromboxane B2 synthesis in blood samples. COX-2 activity was determined by measuring prostaglandin E2 synthesis in blood samples incubated at $37°C$ for 24 h in the presence of lipopolysaccharide. The data were fitted to Hill plots, and the slope ($\beta$) and $IC_{50A}$ values were calculated. For prostaglandin PGE2 inhibition, $\beta$ was estimated to be 0.896, and the $IC_{50A}$ value was estimated to be $0.1454 \mu M$ (51 ng/ml); corresponding values for thromboxane B2 were 1.024 and 1.215 $\mu M$ (427 ng/ml). Through the use of these parameters kindly provided by Novartis, the $I_{\text{max}}$ model was used to estimate the extent of COX-1 and COX-2 inhibition corresponding to our in vivo estimated $IC_{50A}$ value.

Statistical Analysis. Metrological performances of the different end points were assessed using the following statistical model (Systat 10; Systat Software, Inc., San Jose, CA)

$$Y_{ij.k} = \mu + A_i + D_j + T_{tk} + A_i*D_j + A_i*T_{tk} + A_i*T_{jk} + \epsilon_{ijk}$$

(7)

where $Y_{ij.k}$ is the end point value for animal $i$ at day $j$ and time $k$, $\mu$ is the overall mean, $A_i$ is the animal factor, $D_j$ is the day factor, $T_{tk}$ is the measurement time factor, $C*D_{ij}$, $C*T_{jk}$, and $D*T_{jk}$ are the corresponding interactions, and $\epsilon_{ijk}$ is the residual error for $i = 1$ to 8, $j = 1$ to 5, and $k = 1$ to 3.

All of the variables in this model were considered as fixed factors, and a factor was considered significant when $p < 0.05$.

An analysis of variance was used to test the significance of the different factors and to calculate the repeatability of measurements (as a coefficient of variation) using the residual of the model:

$$\text{Repeatability: } CV\% = \frac{\sqrt{\text{Mean square of residual error}}}{\text{mean}} \times 100$$

(8)

Repeatability is the highest level of precision obtained in a given dog for a given day and a given measurement time. Reproducibility (as a coefficient of variation) was calculated based on all of the values recorded during the five days of the study:

$$\text{Reproducibility: } CV\% = \frac{SD}{\text{mean}} \times 100$$

(9)

Reproducibility is always higher than repeatability and is the lowest level of precision encompassing all of the factors of variability in a measurement.

The PK and PD results are presented as mean or median and S.D. For the half-lives, the harmonic mean was calculated. An ANOVA analysis was used to test the significance of the difference between different $IC_{50A}$ values.

Results

Metrological Performance of the PD End Points (Repeatability and Reproducibility). The results are summarized in Table 2. The dog factor was highly significant for all of the end points ($p < 0.001$, data not shown), reflecting the interanimal variability and justifying a crossover design to assess the dogs’ responses in both placebo and test conditions. For several end points, there were significant animal per day interactions, meaning that the day-to-day variation that could be observed for an end point measurement did not follow a systematic trend as would have happened if a “training” effect had occurred during our validation tests. The measurement time (within day) effect was significant for the

$$\text{Effect} = \text{Baseline} \times \left(1 - \frac{I_{\text{max}} \times \text{Dose}^n}{ED_{50} + \text{Dose}^n}\right)$$

(10)
distribution scaled by the unknown bioavailability) was low (6.33 ± 890.2 ± 166.4 ng/ml) was attained at 5.97 ± 1.73 h after administration, and the harmonic mean of the apparent half-life of absorption was 1.32 ± 0.59 h. The harmonic mean of the terminal half-life was relatively high at 21.26 ± 4.83 h.

Time Development of the Kaolin Paw Inflammation Model with and without Meloxicam Administration. Figure 3 illustrates the time course and magnitude of the inflammatory response for the six investigated endpoints in both placebo and treated conditions. The end point values reached maxima 22 and 24 h after the kaolin injection. For the placebo period (without meloxicam), body temperature, creeping time, and paw withdrawal time still were altered 56 h after kaolin administration (spontaneous improvement was <50%, a 100% improvement meaning return to the prekaolin administration condition). Paw edema (measured by the paw circumference) disappeared slowly, a delay of 15 days being necessary to reduce the kaolin-induced edema by approximately 50%. The vertical force exerted by the hind limb still was decreased by approximately 50% on the 10th day after the kaolin administration, indicating a rather sustained inflammation that enables NSAID effects to be followed over a prolonged time period. However, the natural time course of the inflammation was not stable enough to be ignored when modeling the NSAID effect, and a crossover design including a placebo period was mandatory to assess the net effect of the NSAID (see Discussion). In terms of animal welfare, it was observed that for the six investigated endpoints an improvement superior to 85% was observed after kaolin administration between the 14th and 17th days (body temperature), the 24th and 35th days (paw circumference), the 17th and 21st days (lameness score), the 10th and 14th days (creeping time), the 10th and 14th days (vertical force of the paw exerted on the ground), and the 7th and 10th days for the allodynia as assessed by paw withdrawal time.

In treated conditions (after a subcutaneous injection of meloxicam at a dose of 0.2 mg/kg), a clear-cut drug response was obtained for all but one end point, namely, paw circumference. The unresponsiveness of paw circumference to meloxicam was expected (see Discussion) and was not considered for further analysis. The mean time of the peak response occurred for all of the end points between 4.6 and 7.3 h after meloxicam administration. The mean time of the maximum decrease in body temperature was slightly shorter (4.6 h) than the mean time of the peak response for other end points. The mean paw withdrawal time obtained 6 h after meloxicam administration (16.54 ± 6.44 s) was similar to the mean baseline withdrawal time without inflammation (16.79 ± 3.03 s), indicating that complete suppression of allodynia was achieved with 0.2 mg/kg meloxicam. The body temperature, lameness score, creeping time, and vertical force of the paw values did not return to baseline values, the maximal percentages of improvement being of 70, 59, 79, and 66%, respectively. An average duration of drug response was observed for approximately 20 h for all of the end points. After completion of the meloxicam action, the time course of the inflammation did not return to the one observed at the same time during the placebo period, but rather there was for all of the measured end points an overshoot from the 21st hour after meloxicam administration and the following 2 or 3 days.

**TABLE 2**

Repeatability and reproducibility of the selected endpoints

<table>
<thead>
<tr>
<th>Measure</th>
<th>Repeatability CV</th>
<th>Reproducibility CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body temperature</td>
<td>0.41</td>
<td>0.5</td>
</tr>
<tr>
<td>Circumference of the paw</td>
<td>0.44</td>
<td>3.2</td>
</tr>
<tr>
<td>Creeping time under a tunnel</td>
<td>4.66</td>
<td>24.2</td>
</tr>
<tr>
<td>Vertical normalized maximal force of the paw</td>
<td>3.26</td>
<td>7.1</td>
</tr>
<tr>
<td>Paw withdrawal time to a heat stimulus</td>
<td>15.14</td>
<td>20.1</td>
</tr>
</tbody>
</table>

**Fig. 2.** Spaghetti plot of meloxicam plasma concentration (ng/ml) versus time in eight dogs after a subcutaneous administration of meloxicam (0.2 mg/kg).

body temperature. This observed trend was interpreted as reflecting an endogenous circadian rhythm, the body temperature in the afternoon always being higher than that in the morning. This possible confounding factor was not considered in our PK/PD modeling because the absolute difference associated with this factor was only 0.1°C.

Repeatability (i.e., variability within a given measurement time) was appropriate for all of the end points (<5% for the measures of creeping time and vertical normalized maximal force and <0.5% for measures of body temperature and paw circumference). For the withdrawal time measuring analgesia, the CV of repeatability was 15.1%, which is low considering the expected amplitude of the kaolin and meloxicam effect. Reproducibility (i.e., the overall variability between dogs, days, and measurement times) was <5% for the measures of body temperature and paw circumference, <10% for the measure of the vertical normalized maximal force, and <25% for the creeping time and the paw withdrawal time. The animal factor was the main contributor to the overall reproducibility, thus justifying the use of a crossover design for this kind of investigation rather than a parallel design.

**PK Results.** The meloxicam plasma concentration profile after subcutaneous administration of 0.2 mg/kg was interpreted as a monocompartmental model with a first-order rate constant of absorption and a lag time (Fig. 2). The apparent total plasma clearance (Cl/F, clearance scaled by the unknown bioavailability) was low (6.33 ± 1.95 ml h⁻¹ kg⁻¹), and the apparent volume of distribution (V/F, volume of distribution scaled by the unknown bioavailability) was small 192.1 ± 34.4 ml/kg. Peak meloxicam plasma concentration (890.2 ± 166.4 ng/ml) was attained at 5.97 ± 1.73 h after administration, and the harmonic mean of the apparent half-life of absorption was 1.32 ± 0.59 h. The harmonic mean of the terminal half-life was relatively high at 21.26 ± 4.83 h.
**PK/PD Analysis.** Figure 4 shows the fit of the responses for all of the end points (body temperature, lameness score, creeping time, vertical force of the paw, and paw withdrawal time) for a representative dog. For the eight dogs investigated and the five measured end points, a successful fit was obtained for 34 of the 40 recorded time courses.

Table 3 gives the mean PD parameters for all of the end points. Mean values of IC$_{50A}$/ for the meloxicam effect on kaol(t) ranged from 210 ng/ml for body temperature to 546 ng/ml for the vertical force measured by the force plate. The IC$_{50A}$/ value for body temperature was lower than the other IC$_{50A}$/ values, but the differences were not significant.
The interanimal variability of the estimated IC50A value was estimated using a coefficient of variation that ranged from 28 (for analgesia) to 86% (creeping time) with intermediary values for the other end points. The meloxicam sensitivity (i.e., the slope of the concentration-effect relationship) was relatively high for all of the end points (from 2.56 to 4.37), indicating that the effect was rapidly reaching Imax with increasing meloxicam plasma concentrations.

TABLE 3
Estimated mean pharmacodynamic parameters describing meloxicam anti-inflammatory, analgesic, and antipyretic effects after a subcutaneous administration of meloxicam at a dose of 0.2 mg/kg to eight dogs

<table>
<thead>
<tr>
<th>Units</th>
<th>Definition</th>
<th>Body Temperature</th>
<th>Lameness Score</th>
<th>Creeping Time</th>
<th>Force Plate</th>
<th>Analgesia</th>
</tr>
</thead>
<tbody>
<tr>
<td>kin</td>
<td>Zero-order rate constant for production of the response</td>
<td>94.6 (14)</td>
<td>0.0057 (25)</td>
<td>13.8 (57)</td>
<td>7.64 (119)</td>
<td>43 (63)</td>
</tr>
<tr>
<td>IC50A</td>
<td>Plasma meloxicam IC50 on the time development of kaolin inflammation</td>
<td>69 (51)</td>
<td>56 (28)</td>
<td>72 (27)</td>
<td>68 (37)</td>
<td>61 (28)</td>
</tr>
<tr>
<td>n2</td>
<td>No unit</td>
<td>2.61 (51)</td>
<td>1.87 (23)</td>
<td>2.91 (83)</td>
<td>2.27 (74)</td>
<td>1.13 (77)</td>
</tr>
<tr>
<td>koff</td>
<td>First-order rate constant for loss of response</td>
<td>2.51 (14)</td>
<td>4.99 (49)</td>
<td>3.45 (63)</td>
<td>3.03 (123)</td>
<td>5.37 (45)</td>
</tr>
<tr>
<td>ImaxA</td>
<td>Maximal possible clinical effect (between 0 and 1)</td>
<td>0.84 (11)</td>
<td>0.82 (16)</td>
<td>1 (N.A.)</td>
<td>1 (N.A.)</td>
<td>1 (N.A.)</td>
</tr>
<tr>
<td>IC50A</td>
<td>Plasma meloxicam concentration for ImaxA/2 for the clinical endpoints</td>
<td>210 (64)</td>
<td>466 (55)</td>
<td>400 (86)</td>
<td>546 (64)</td>
<td>390 (65)</td>
</tr>
<tr>
<td>n1</td>
<td>No unit</td>
<td>4.37 (66)</td>
<td>3.93 (85)</td>
<td>2.56 (91)</td>
<td>3.03 (95)</td>
<td>3.88 (105)</td>
</tr>
<tr>
<td>P5</td>
<td>Same as koff</td>
<td>5.10 (35)</td>
<td>24.3 (46)</td>
<td>32.8 (42)</td>
<td>303 (125)</td>
<td>483 (56)</td>
</tr>
<tr>
<td>P5</td>
<td>Parameters characterizing time</td>
<td>0.023 (47)</td>
<td>0.0038 (100)</td>
<td>0.015 (86)</td>
<td>0.0052 (130)</td>
<td>0.0112 (101)</td>
</tr>
<tr>
<td>P5</td>
<td>development of kaolin inflammatory</td>
<td>0.96 (20)</td>
<td>1.05 (10)</td>
<td>0.94 (16)</td>
<td>0.71 (72)</td>
<td>0.95 (21)</td>
</tr>
</tbody>
</table>

N.A., not applicable.
In the current modeling, meloxicam developed a second action through a modulation of kai(t). The estimated IC_{50B} values for this secondary effect were systematically lower than the corresponding IC_{50A} values of the main effect of meloxicam [mean IC_{50A}/IC_{50B} ratio from 6 (for analgesia) to 8 (for vertical forces)]. The IC_{50B} value expressed the potency of meloxicam to delay the disappearance of kaolin inflammation, whereas the IC_{50A} value expressed the potency of meloxicam to mitigate the clinical expression of the inflammation (see Discussion).

Mean PK and PD parameters were used to simulate meloxicam dosage regimens ranging from 0.05 to 2 mg/kg for body temperature, lameness score, vertical force on a force plate, and paw withdrawal time (Fig. 5). Considering the maximal simulated responses, the dose-response relationships were modeled (Fig. 6), and the estimated ED_{50} values were calculated for the different end points. The ED_{50} value was 0.0507 mg/kg for body temperature and from 0.091 to 0.127 mg/kg for the other end points.

The whole-blood ex vivo assay is useful for predicting the clinical relevance of given levels of COX-1 and COX-2 inhibition (Fig. 7). For the present experiment, it was calculated that the plasma concentration corresponding to our estimated in vivo IC_{50A} value would ensure ex vivo an inhibition from approximately 80 (body temperature) to 90% (others end points) of the COX-2 isoenzyme and from 32.6 to 52.9% of the COX-1 isoenzyme (Table 4). The in vivo COX-2 versus COX-1 selectivity, as evaluated by the ratio of COX-2/COX-1 inhibition for a plasma concentration corresponding to the in vivo IC_{50A} value, was from 1.72 to 2.46 depending on the end point. The in vitro IC_{50} value (51 ng/ml) versus the in vivo IC_{50A} ratio was from 4.1 to 10.7 depending on the end point.

**Fig. 5.** Simulated values of four investigated end points after a single subcutaneous meloxicam administration at 0 (placebo), 0.055, 0.1, 0.2 (thick line), 0.3, 0.5, and 2 mg/kg b.wt. A–D, body temperature (°C) (A), lameness score (B), vertical force exerted by the paw expressed as a percentage of the maximal observed response to kaolin (C), and paw withdrawal time expressed as a percentage of the maximal observed response to kaolin to a heat stimulus (D). Kaolin and meloxicam were administered at 24 and 48 h, respectively. The presence of a rebound (compared with the placebo period) is clearly seen for the four end points.

**Fig. 6.** Dose-response relationships for meloxicam. The effects (body temperature (—), withdrawal time (••••) of the paw as a measure of analgesia, and lameness score (—)••••) were simulated with the PK/PD model using mean PK and PD parameters; the relationship between the dose of meloxicam (mg/kg) and the maximal response obtained for each dose were fitted with a inhibitory sigmoidal model (see eq. 8 in the text).
corresponding to the different estimated in vivo IC₅₀ values.

Fig. 7. Ex vivo COX-1 and COX-2 inhibition in nine dogs. Relative change (0–1) against log₁₀ blood concentrations of meloxicam in nine dogs of thromboxane B₂ as an index of COX-1 inhibition and prostaglandin E₂ as an index of COX-2 inhibition (Novartis file; for details, see King et al., 2010).

### TABLE 4

<table>
<thead>
<tr>
<th>Parameter</th>
<th>%COX-2 Inhibition</th>
<th>%COX-1 Inhibition</th>
<th>In Vivo Selectivity</th>
<th>Ex Vivo IC₅₀</th>
<th>In Vivo IC₅₀A</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body temperature</td>
<td>80.1</td>
<td>32.6</td>
<td>2.46</td>
<td>4.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lameness score</td>
<td>89.8</td>
<td>52.2</td>
<td>1.72</td>
<td>9.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Creeping time</td>
<td>88.4</td>
<td>48.3</td>
<td>1.83</td>
<td>7.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Force plates</td>
<td>91.2</td>
<td>52.9</td>
<td>1.73</td>
<td>10.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Analgesia</td>
<td>88.1</td>
<td>47.7</td>
<td>1.85</td>
<td>7.6</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Discussion

The main motivation of the present study was to develop a humane inflammation model for preclinical PK/PD investigations in dogs. The dog is a standard species for the preclinical development of drugs, and its use should be ethically acceptable. In a European Union context, this feature for an animal model is now critically scrutinized, and a fully reversible inflammatory model is considered as desirable because it allows dogs used in this type of trial to be reallocated after the completion of the study to other protocols or, in our case, to be definitively rehabilitated as companion animals. Kaolin was selected as a phlogistic agent because it acts mainly as an inert foreign body. In rats, kaolin does not activate the complement cascade (Noordhoek et al., 1977) but rather triggers the release of kinins and prostaglandins (Lewis, 1978). When administered outside an articulation, it is eliminable through sterile abscess formation, an inflammation resolution that guarantees a lack of delayed side effects. This is not the case for inflammation models having an immunological component such as the classic Freund adjuvant arthritis model that leads to the development of an irreversible osteoarthritis in dogs. In addition, we recently showed that the subcutaneous injection of kaolin into a cat’s paw produced a well defined, reproducible, and reversible inflammatory response (Giraudel et al., 2005b), and this model was found to be suitable for studying simultaneously the analgesic, anti-inflammatory, and antipyretic effects of NSAIDs.

From the present trials, it can be concluded that the kaolin model in dogs is ethically acceptable. It mimics the natural process of abscess formation and maturation, and it was fully reversible within 8 weeks. We now use this model routinely, and we rarely observe side effects such as paw infection (one case for 40 dogs), but limited skin necrosis is a more frequent finding in this model.

To be useful for preclinical investigations of NSAIDs, a canine inflammatory model should be able to predict the order of magnitude of a future dosage regimen in different target species including man. To assess this property, we selected meloxicam as a test NSAID because it has been used extensively as a therapeutic agent in both animals and humans and its dosage regimen is now well established in both man and dogs (Engelhardt, 1996; Türck et al., 1996; Busch et al., 1998; Slingsby and Waterman-Pearson, 2000; Lascelles et al., 2001). In addition, the PK profile of meloxicam was known to be very similar in man (Davies and Skjodt, 1999) and dogs (Busch et al., 1998). This has been confirmed in the present study where the plasma clearance determined in man (6.1 ml/kg per hour) can be considered as practically equal to the clearance after intravenous administration in man (6.1 ml/kg per hour for a body weight of 70 kg) (Türck et al., 1996). Only one meloxicam dose was investigated due to our two-way crossover design for two hind legs with a control and a test period, but the selected dose was likely high enough to sweep all of the concentration-effect relationship, allowing us to properly estimate PD parameters.

The main limit of this model is the lack of a prolonged steady inflammation over several days to enable a NSAID to be tested on a stable inflammation baseline. Indeed, the price to pay for a reversible inflammatory model is to have inflammation with a rapid time course, increasing over 2 to 3 days and then decreasing immediately over the next few days (i.e., without a well defined plateau). The practical consequence of this is the necessity to characterize precisely the time development of the inflammation progression during a placebo period for each tested dog and to model simultaneously the placebo and the test period to take into account the confounding factor of the natural time development of kaolin inflammation. This is what we have carried out with the present modeling approach. Several models of disease progression were proposed (for an extensive review, see Mould, 2007). In this study, we selected a simple Bateman function describing the transient effect of kaolin. In fact, the time course of the kaolin inflammation was not the same during the placebo and test periods. For several end points, we observed a re-
bound effect (i.e., that after cessation of meloxicam action the levels of paw inflammation were greater than those obtained at the same time after placebo dosing). A possible explanation for this delaying action of meloxicam on the disappearance of the inflammation is the classically reported impeding action of NSAIDs on abscess formation that is required to physically eliminate the administered kaolin. Therefore, we took into account in our mathematical model this effect of meloxicam on the progression of the inflammation by modulating the actual value of $P_2$ (see eq. 4), the parameter reflecting the rate of inflammation disappearance. $P_2$ was modulated by the actual meloxicam concentration through a Hill inhibitory function (see eq. 7). This part of our model predicted that for a high meloxicam plasma concentration the time development of the kaolin action was slowed down temporally or even stopped. It should be noted that our model is not equivalent to classic paw edema as observed in rats after the intra-articular administration of a phlogistic agent. In our case, the increase in the paw volume was associated with lymph node hypertrophy (popliteal), which can be interpreted as a lymphatic drainage blockade protecting the animal from systemic exposure to kaolin particles. This explains why meloxicam (as other NSAIDs) has no short-term effect on the paw diameter. As an alternative to this model, we also explored a precursor-dependent indirect PD response model, as outlined by Sharma et al. (1998), to describe tolerance and rebound phenomena. This model fit our data well and also showed that this model would be useful to investigate dose-effect relationships of NSAIDs and also could be relevant for in vitro to in vivo extrapolations. The limitation of the model is the requirement of a rather advanced PK/PD analysis to accurately estimate the PD parameters of interest.

Acknowledgments

We thank Jean-Pierre Gau and Simone Baurés for skilled technical assistance.

Authorship Contributions

Participated in research design: Jeunesse, C. Toutain, Letellier, Giraudel, and P. Toutain.

Conducted experiments: Jeunesse, Bargues, and C. Toutain.

Contributed new reagents or analytic tools: Lacroix.

Performed data analysis: Jeunesse and P. Toutain.

Wrote or contributed to the writing of the manuscript: Jeunesse, Letellier, and P. Toutain.

References


Address correspondence to: Pierre-Louis Toutain, UMR1331 Toxalim Physiologie, École Nationale Vétérinaire de Toulouse, 23 Chemin des Capelles, BP87614, 31076 Toulouse cedex 03, France. E-mail: pl.toutain@envt.fr