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RESEARCH PAPER

Population Pharmacokinetic Analysis of Blood and Joint Synovial Fluid Concentrations of Robenacoxib from Healthy Dogs and Dogs with Osteoarthritis

Hanna E. Silber • Claudia Burgener • Ingrid M. Letellier • Mathieu Peyrou • Martin Jung • Jonathan N. King • Philippe Gruet • Jerome M. Giraudel

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ABSTRACT

Purpose The purpose of this population analysis was to characterize the pharmacokinetic properties of robenacoxib in blood and stifle joint synovial fluid of dogs.

Methods Data were obtained from two studies: 1) 8 healthy Beagle dogs in which an acute inflammation was induced by injection of urate crystals into one joint; 2) 95 dogs from various breeds diagnosed with osteoarthritis (OA). Robenacoxib concentrations in blood and synovial fluid were measured using a validated HPLC-UV and LC-MS method. Non-linear mixed effects modeling was performed using NONMEM6.

Results A two-compartment pharmacokinetic model with linear elimination was developed to describe blood concentrations of robenacoxib. Blood clearance in healthy animals was found to be 75% higher than in OA dogs. Synovial fluid concentrations were modeled using an effect-compartment-type model predicting longer residence times in OA dogs compared to healthy Beagles (*e.g.* concentrations above the IC_{50} for COX-2, respectively, 16 h *vs.* 10 h at 1.5 mg/kg).

Conclusions Robenacoxib was found to reside longer at the effect site (inflamed joint) compared to blood in both healthy and OA dogs. These results may explain the good efficacy observed with once-daily dosing in clinical trials and the high safety index of robenacoxib in dogs.

KEY WORDS COXIB · dog · robenacoxib · tissue selectivity

ABBREVIATIONS

BLQ	below limit of quantification
COX	Cyclooxygenase
COX-1	Cyclooxygenase-1
COX-2	Cyclooxygenase-2
FOCE	first-order conditional estimation method
HPLC-UV	high pressure liquid chromatography with ultraviolet detection
IIV	inter-individual variability
IOV	inter-occasion variability
LC-MS	liquid chromatography with mass spectrometry
LLOQ	lower limit of quantification
MTT	Mean Transit Time
N	number of transit compartments
NSAID	non-steroidal anti-inflammatory drug
OA	osteoarthritis
OFV	objective function value
VICH	international cooperation on harmonisation of technical requirements for registration of veterinary medicinal products
VPC	visual predictive check

H. E. Silber
Novartis Pharma AG, Modeling & Simulation Pharmacology
Postfach CH-4002 Basel, Switzerland
e-mail: hanna.silber@novartis.com

C. Burgener • M. Peyrou • M. Jung • J. M. Giraudel (✉)
Novartis Centre de Recherche Santé Animale SA
CH-1566 Saint-Aubin, Switzerland
e-mail: jerome.giraudel@novartis.com

I. M. Letellier • P. Gruet
Novartis Santé Animale SAS
FR-92500 Rueil-Malmaison, France

J. N. King
Novartis Animal Health Inc.
CH-4058 Basel, Switzerland

INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs) are the most widely used analgesics in veterinary and human medicine. In addition to inhibition of pain, NSAIDs also inhibit inflammation and fever. In dogs, the main clinical

uses of NSAIDs are for the management of pain and inflammation associated with surgery and osteoarthritis (OA). The beneficial effects of NSAIDs in reducing pain, inflammation and fever are largely mediated by the inhibition of cyclooxygenase-2 (COX-2), which is the COX isoform responsible for the generation of pro-inflammatory prostaglandins (1). In contrast, the COX-1 isoform is largely responsible for the production of prostaglandins with protective functions, notably in the gastrointestinal tract. As a result of intense research efforts, several COXIBs which are COX-2 selective were marketed in the last decade in humans and in dogs. These drugs have been shown to have reduced incidences of blood loss following surgical interventions and gastrointestinal toxicity during long-term use, compared to older non-selective NSAIDs, but are not devoid of all side effects as originally hoped (1,2). COXIBs remain, nevertheless, an interesting class of NSAIDs, with a favorable risk-benefit ratio, especially in cats and dogs which are especially sensitive to non-selective NSAIDs and in which an increased risk of cardiovascular side effects with NSAIDs has not been reported so far.

Robenacoxib is a novel and highly selective inhibitor of COX-2 in cats and dogs (3–5) and is now available in several European countries for the treatment of pain and inflammation in cats and dogs (Onsior® injection and tablets). Robenacoxib shows more than one-hundred-fold selectivity for COX-2 compared to COX-1 in dogs *in vitro* using the whole blood assay (5). Although it has a short blood half-life (approximately 1 h) in dogs (6), robenacoxib has demonstrated good efficacy in field trials when given once daily (7,8). Robenacoxib also has a high safety index in dogs, with no relevant toxicity detected in target animal safety studies with daily dosages as high as 40 mg/kg for one month and 10 mg/kg for 6 months (9). It is a weakly acidic compound and is highly bound to proteins, with a similar structure to lumiracoxib (10). Since lumiracoxib and other acidic NSAIDs have demonstrated higher concentrations and longer residence times in inflammatory exudate compared to blood (11,12), it was hypothesized that robenacoxib would display similar tissue selectivity as other weakly acidic NSAIDs. In a tissue cage model in rats, average concentrations were 2.9 times higher, and the mean residence time was 3 times longer (15.9 h *versus* 5.3 h) for robenacoxib in inflammatory exudate compared to blood (4). However, tissue cages are only models for joints, and robenacoxib is used clinically in cats and dogs. It was therefore important to confirm these results in the inflamed stifle synovial fluid of the target species.

The objective of the present study was therefore to characterize the pharmacokinetic properties of robenacoxib in blood and synovial fluid of dogs using a population analysis. Our hypothesis was that robenacoxib would display tissue-selective pharmacokinetics in dogs leading to higher concentrations and a longer residence time in inflamed joint synovial fluid compared to blood.

MATERIALS AND METHODS

Animal Phase of the Study in Healthy Beagles

This study was conducted at the Novartis Centre de Recherche Santé Animale SA, Saint-Aubin, Switzerland under an animal experimentation permit obtained from the Swiss cantonal authorities. Eight Beagle dogs weighing between 10.2 to 14.3 kg and aged between 7 and 13 years were housed and handled according to site SOPs. The dogs were healthy and devoid of any detected orthopedic disorder as established by clinical assessments and orthopedic examinations. They were fasted for at least 8 h prior to and 4 h after dosing. On the day of NSAID administration, they were injected in the intra-articular space of the right stifle joint with a suspension of monosodium urate crystals (15 mg of dry urate crystals, Fluka n° 51449) in physiological saline (2 ml, NaCl 0.9%) according to a previously described method (13). For the purpose of the injection of the urate crystals, the animals were sedated with medetomidine, and the sedation was reversed with atipamezole. This procedure induces an acute and severe but reversible arthritis, known as the urate-induced synovitis model. After injection, the dogs progressively become lame and finally put no weight on the affected limb 2 to 3 h after the injection, with a spontaneous recovery and a gate back to normal 24 h after the induction of the inflammation. Robenacoxib (one to two non-flavored lactose-based tablets containing 20 mg robenacoxib resulting in an administered dose of 2.27 to 2.67 mg/kg) was administered to the dogs 3 h after the injection of the urate crystals. The dogs were then sampled repeatedly for blood and synovial fluid. The study consisted of 4 periods with a washout of at least 3 weeks between phases, and during each period each dog was first injected with urate crystals and then administered a single dose of robenacoxib. Following each of these administrations, 3 or 4 blood samples (4 in period 1 and 3 in periods 2, 3 and 4) were taken by venipuncture from a jugular vein at pre-determined time points (0.5, 1, 2, 3, 4, 5, 6, 8, 10 and 12 h post-treatment). Synovial fluid samples were collected from the right (inflamed) and left (non-inflamed) stifle at similar pre-determined time points (1, 3, 4, 5, 8, 10 and 12 h post-treatment). The time points for synovial fluid collection were always at least 7 h apart, and 1 or 2 samples were collected from each stifle (2 samples in periods 1, 2 and 4 and 1 sample in period 3) after each administration in order to have for all dogs a synovial fluid concentration value at each of the pre-determined time

points. These samples were collected under sedation (medetomidine and propofol) and under strict aseptic conditions via a medial arthrocentesis using 23 G needles and 2 ml syringes. Out of the 112 planned synovial fluid samples, 102 could be collected and processed for sample analysis. Among the 102 analyzed samples, 84 were not or were only very slightly contaminated with blood, and for the other 18 the contamination was judged to be low enough for the results to be incorporated in the dataset. For the 10 remaining collections, the sampling was not successful, or the collected amount was not sufficient for the analysis.

Animal Phase of the Study in Dogs with Osteoarthritis

This study was authorized by the French authorities (AFSSA) and carried out in compliance with Good Clinical Practice (VICH Guideline 9). It was a multi-center field study (20 veterinary clinics in different geographical locations in France) with the objective to evaluate the pharmacokinetics of robenacoxib in blood and stifle joint synovial fluid in dogs suffering from OA after multiple (for 7 days) and single oral administration of the drug. The inclusion criteria were as follows: dogs with clinical signs of stifle OA as evidenced by the presence of lameness and/or pain of at least 3 weeks duration and confirmed by X-ray, older than 6 weeks and with a body weight between 10 and 80 kg. A thorough investigation of the animals' clinical condition supported by blood biochemical and hematological analyses was performed by the investigator, and an owner's consent form was completed and signed before the dogs could enter the study. The exclusion criteria were as follows: lameness associated with neoplasia, lameness associated with a primary neurological disorder, surgery of the selected knee joint in the previous 30 days, absence of radiographic evidence of knee OA, animals intended for breeding or known to be pregnant or lactating, dogs with severe concomitant disorders (*e.g.* kidney, liver or gastrointestinal tract), dogs which received local or systemic NSAIDs within 14 days prior to their inclusion in the study, dogs which received corticosteroids within 30 days (systemic or intra-articular) prior to their inclusion in the study. Included dogs were allocated to one of two treatment groups according to the timing of their recruitment. At the beginning of the recruitment period, all dogs were included in Group 1 (7 days of treatment at 1–2 mg/kg/day robenacoxib). Once all dogs from Group 1 were recruited (the protocol stated a minimum of 36 cases for both groups), subsequently recruited dogs were included in Group 2 (single dose administration at 1–2 mg/kg robenacoxib). Within each treatment group, dogs were allocated randomly to a sampling group defined by the post-administration synovial fluid sampling time, and this randomization was stratified according to the investigation center. One set of numbered envelopes from 1 to 9 was prepared for each investigation center. Each envelope contained the synovial fluid sampling time allocated to the dog (0.5, 1, 2, 3, 5, 7, 10, 16 or 24 h after treatment administration). After inclusion of a dog in the study, the investigator selected the envelope bearing the lowest number and opened it to reveal the sampling time allocated to the dog. In addition to the synovial fluid sample (0.5–1 mL), the investigator also had to collect 5 blood samples (2 mL) from the following time points: 0.25, 0.5, 1, 2, 3, 5, 7, 10, 16 or 24 h after treatment administration. One of these 5 time points had to correspond to the time point of the synovial fluid collection determined by randomization. The administration of robenacoxib preceding the start of the sampling campaign (last administration for Group 1 and single administration for Group 2) was performed by the investigator by gavage in the veterinary clinic. For this administration, all animals were fasted for at least 8 h prior to and 4 h after dosing. The exact times of tablet administration and sampling were recorded for all animals. In total, 47 animals were included in Group 1 and 56 in Group 2, but only 41 and 54, respectively, were included in the population analysis. Reasons for not including 8 dogs in the analysis were that 1) blood concentrations were below the lower limit of quantification (LLOQ) or very low for all time points, which was most likely due to a dosing error, 2) there was a lack of treatment compliance in the first 6 administrations performed by the owner (Group 1), 3) the owner administered a forbidden concomitant treatment, or 4) in one case, the owner did not bring the animal to the clinic on time. The test item administered to the animals consisted of flavored tablets containing 20 mg or 40 mg robenacoxib (Onsior® tablets, Novartis Animal Health, Basel, Switzerland), and resulting doses ranged from 1.03 to 1.99 mg/kg in Group 1 (average dose of 1.47 mg/kg) and 1.03 to 2.00 mg/kg in Group 2 (average dose of 1.42 mg/kg). The mean ± SD age of the dogs was 7.7 ± 3.3 years with a range of 0.7 to 14.3 years. The mean ± SD body weight of the dogs was 33.9 ± 12.5 kg with a range of 10.0 to 72.7 kg. There were no differences between Group 1 (7.9 ± 3.7 years, 33.2 ± 12.7 kg) and Group 2 (7.6 ± 3.1 years, 34.4 ± 12.4 kg) for age ($p = 0.69$) or body weight ($p = 0.59$).

Analytical Method

Blood and synovial fluid samples were collected into tubes containing EDTA and no anticoagulant, respectively, and stored at approximately −20°C prior to analysis. Determination of robenacoxib blood concentrations involved an initial analysis by high pressure liquid chromatography-ultraviolet (HPLC-UV ("UV method")), covering the range of 500–20,000 ng/mL and, if required, a subsequent analysis by liquid chromatography-mass spectrometry (LC-MS ("MS

method")), covering the range of 2–100 ng/mL (6). The analytical method was validated using quality control spiked matrix specimens run with each sequence of unknown samples, and independent of calibration standards. Samples containing robenacoxib in blood were shown to be stable at approximately −20°C for 5 months. The LLOQ of the analytical method in blood was 2 ng/mL.

The same method was used for the synovial fluid with the following exceptions: 1) only the "MS method" was used; 2) only 0.1 mL synovial fluid+0.4 mL water were extracted (instead of 0.5 mL blood, the sensitivity was therefore reduced and the calibration levels were 10, 15, 25, 50, 100, 250, 500 ng/mL (instead of 2, 3, 5, 10, 20, 50, 100 ng/mL) resulting in an LLOQ of 10 ng/mL); 3) the solid phase extractions often required manual pushing with nitrogen from a hose because of the viscous nature of the synovial fluid.

Pharmacokinetic Model Development

Model development was performed in two steps. In the first step, blood concentration time data were analyzed for the two populations. In the second step, the joint concentration observations were added, and the model parameters describing the blood concentration time profile were fixed to the final values.

In step one, 2- and 3-compartment models were evaluated for the description of the distribution and elimination of robenacoxib. Different models were evaluated for the description of the absorption phase including first-order absorption, a combination of first and zero-order absorption, the inclusion of a lag-time and a transit-compartment model (14).

In the second step, the joint concentration-time data were included in the model. To increase the model stability, and to reduce the run times, the first part of the model describing the blood concentration time profiles was kept fixed to the final estimates of that model. The joint data were modeled using an effect-compartment model, which was here used to describe the time-lag between the blood and the joint concentrations (15). The model further assumes that the amount of drug transferred to the effect compartment (joint) does not affect the blood concentrations. This assumption was considered reasonable considering the small volume of the joint compared to the overall blood volume of the animals. As observations were available in the effect compartment, the rate in (k_{in}) and out (k_{out}) of the compartment could be estimated separately, thus allowing the joint concentration at steady-state to be different from the blood concentrations, which would not be possible otherwise.

The healthy and the OA dogs were initially assumed to have the same parameters describing the concentration-time profiles, but differences between the populations were then evaluated for their significance for the absorption model and disposition parameters including distribution of robenacoxib to the joint. In addition, differences in the distribution to and from the acutely inflamed (urate model in healthy dogs), the chronically inflamed (OA dogs) joint, and the normal joint were evaluated.

Inter-Individual and Residual Error Model

Inter-individual variability (IIV) was expressed using an exponential model according to Eq. (1):

$$P_i = P \cdot e^{\eta_i} \tag{1}$$

where P_i is the individual parameter estimate determined by P, the population parameter estimate, and η_i, the individual random effect (a zero-mean random variable with the variance ω^2), which accounts for the difference between the population parameter value and the individual parameter value. Correlations between individual random effects parameters were evaluated during model development.

Inter-occasion variability (IOV) was tested on absorption and clearance parameters of the healthy population as the data were collected on four different occasions (16). The IOV was included in the same way as the IIV using kappa (κ) variables according to Eq. (2):

$$P_{im} = P \cdot e^{\eta_i + \kappa_{im}} \tag{2}$$

where P_{im} is the individual parameter value at occasion m, η_i is the individual random effect across occasion and κ_{im} is the occasion-specific individual random effect (both being zero-mean random variables with the variance ω^2 and π^2, respectively).

An additive model was used to describe the residual variability on log-transformed data. This model corresponds approximately to a proportional model on normal data. Different residual error models were evaluated, including proportional and slope-intercept models, as well as including a higher residual error during the absorption phase and adding individual variability in the residual term (17). In addition, separate residual variability terms were used for the different studies and for the blood and joint measurements.

Inclusion of Data Below the Limit of Quantification

Different approaches for inclusion of data below the limit of quantification (BLQ) were evaluated, including setting the values BLQ to half the lower limit of quantification (LLOQ/2), also known as the M2 method (18). When the M2 method was used, the first observation in each individual, which was BLQ during the elimination phase,

was set to LLOQ/2, and the subsequent observations were discarded. If a series of observations BLQ were observed during the absorption phase, the last of these values was kept instead.

The M3 method, which estimates the likelihood that the BLQ observations are below the LLOQ, was also evaluated as this method has been shown to give less biased parameter estimates compared to the M2 method (18). For this method, all observations BLQ were kept in the data set.

Data Analysis and Model Evaluation

Non-linear mixed-effects modeling of the data generated in the two studies was performed using NONMEM version VI (GloboMax, Hanover, Md). The first-order conditional estimation method (FOCE) was used for all analyses in combination with the Laplacian method for categorical data when needed (for analysis of BLQ observations using the M3 method). The interaction (INTER) option was added when appropriate (for analysis with a proportional error or inter-individual variability in the residual error).

Model selection was based on statistical significance between competing models using the objective function value (OFV) obtained from NONMEM, graphical assessment and validity of parameter estimates. The OFV is proportional to -2*logLikelihood of the data given the model, and a lower value indicates a better model. The difference between two nested models is approximately χ^2-distributed, and a difference in OFV of 3.84 corresponds to $p<0.05$ for one degree of freedom. Graphical assessment was performed using the R-based software Xpose version 4.1 (19).

The final model was evaluated using internal validation procedures. Precision of parameter estimates was assessed using a bootstrap with 200 samples from which the standard errors of the parameter estimates were calculated.

A visual predictive check (VPC) was performed to evaluate the predictive properties of the model based on 1000 simulated data sets.

Model Predictions of Different Doses

Data from the final model described above were used to predict steady-state concentrations of robenacoxib in blood and inflamed synovial fluid at doses of 1, 1.5 and 2 mg/kg robenacoxib, representing the minimum, median and maximum of the clinically recommended dose of robenacoxib for dog OA (1–2 mg/kg). The concentrations in the typical individual *versus* time data were then compared to the concentrations of robenacoxib required to inhibit the COX isoforms as surrogate markers for efficacy ($IC_{50,cox2}$; $IC_{80,cox2}$) and safety ($IC_{20,cox1}$). Values of $IC_{50,cox2}$ (14.04 ng/mL), $IC_{80,cox2}$ (77.01 ng/mL) and $IC_{20,cox1}$ (92.97 ng/mL) were obtained from a previous study (5). The Model 1 parameters computed in this study (*i.e.* using the so-called two-stage approach) were selected because these computations required fewer assumptions than Model 2 computations.

RESULTS

Blood Pharmacokinetics

A two-compartment linear model was found to describe well the disposition of robenacoxib in blood. The final model is shown in Fig. 1, and parameter estimates are presented in Table I. The population prediction of the blood and the joint concentrations in healthy and OA dogs are shown in Figs. 2 and 3, respectively. Basic goodness-of-fit graphics are shown in Figs. 4 and 5. Figs. 6 and 7 show individual predictions of the healthy and the OA dogs, respectively.

The clearance of robenacoxib from blood of healthy dogs was 75% higher compared to dogs with OA.

The oral absorption of robenacoxib in healthy Beagle dogs was fast and could be well described using a first-order absorption model. The absorption was slower and more variable in dogs with OA compared to the healthy dogs, and therefore a different model, the transit-compartment model, was used to describe the absorption in the OA population. This model is more flexible compared to the first-order absorption model, and it also performed better compared to a first-order absorption model with a lag-time.

Inter-individual variability was included for the absorption parameters and CL/F. A combined additive and proportional model for the residual variability on log-transformed data was found to be sufficient. Inter-occasion variability could not be estimated successfully on any parameter where it was evaluated, although it was quite obvious from graphical assessment that variability between occasions was present. The proportional error was estimated as 86 and 73% for the healthy dogs and the OA dogs, respectively. The additive error was fixed to a low value and was mainly included for stability reasons. The failure to properly assess the magnitude of the IOV is a plausible reason for the high residual error in the healthy population.

Drug Distribution to the Joint

The distribution of robenacoxib to the joint was successfully described using a link model. The distribution rate of robenacoxib into the joint (k_{in}) was found to differ between healthy and OA dogs, but it was not possible to differentiate between the normal and the inflamed joint of the healthy

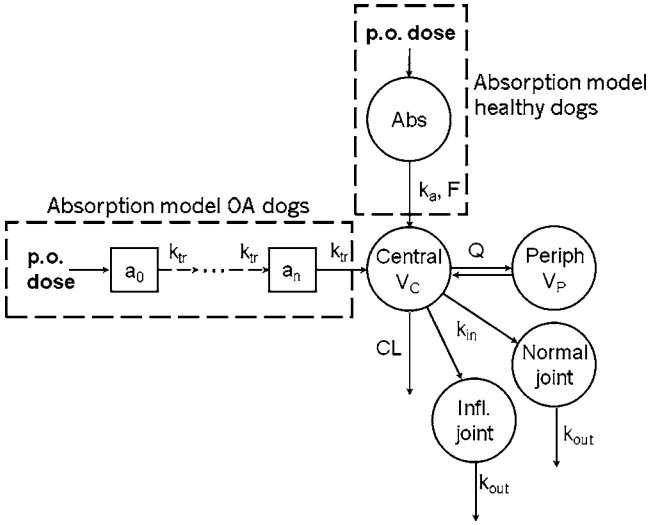

Fig. 1 Diagram of the structural model for robenacoxib concentrations in blood and joint.

dogs. The drug was estimated to enter the joint of the OA dogs 1.8 times faster compared to the healthy dogs. The rate out of the joint (k_{out}) was found to be different between the inflamed and the normal joint, but it was not possible to distinguish between the two populations for the rate out of the inflamed joint. Robenacoxib was estimated to be eliminated from the normal joint 1.54 times faster than from the inflamed joint. Taken together, these results indicate that robenacoxib reaches higher concentrations and remains for a longer time in the inflamed joints in OA dogs compared to both normal and inflamed joints in healthy dogs.

It is possible that the relatively small amount of synovial fluid data (few individuals of the healthy dogs and the sparse sampling of the OA dogs) prevented the differentiation between the normal and inflamed joint of the healthy animals and the differentiation between the acute inflammation of the healthy dogs and the chronic inflammation of the OA dogs.

Inter-individual variability could not be identified for any of the parameters driving the distribution into the joint, possibly due to the sparseness of the joint data. Proportional error for the joint was estimated separately for the two populations and was estimated as 81 and 135% for the healthy and the OA dogs, respectively.

The parameter estimates did not change significantly when the joint samples that were reported to be contaminated with blood were excluded from the data, and these samples were therefore left in the data set.

Data Below the Limit of Quantification

Both the M2 and the M3 methods were evaluated for the handling of data below the LLOQ. The parameter estimates were very similar using both methods, but the M2 method was more stable and was therefore used for the final analysis.

Table I Final Parameter Estimates

	Parameter	Typical value (CV%)	Inter-individual variability,% (CV%)
Absorption	$k_{a\ HA}$[a] (/h), Absorption rate	1.18 (36.7)	26.5 (211)
	N_{OA}[b] (−), Number of transit compartments	3.08 (12.1)	91.2 (32.2)
	MTT_{OA} (min), Mean transit time	1.90 (8.0)	92.7 (20.0)
Distribution in blood	CL/F_{HA} (L/h/kg), Clearance	1.29 (13.1)	15 (191)
	CL/F_{OA} (L/h/kg), Clearance	0.736 (8.0)	15 (191)
	Vc/F (L/kg), Central volume	1.06 (17.2)	–
	Vp/F (L/kg), Peripheral volume	1.12 (71.4)	–
	Q/F (L/h/kg), Inter-compartmental clearance	0.084 (29.7)	–
Distribution to joint	$k_{in\ HA}$ (/h), Rate into joint	0.241 (28.1)	–
	$k_{in\ OA}$ (/h), Rate into joint	0.433 (23.8)	–
	$k_{out\ Inflamed\_joint}$ (/h), Rate out of inflamed joint	0.400 (16.5)	–
	$k_{out\ Normal\_joint}$ (/h), Rate out of normal joint	0.615 (16.7)	–
Residual error	Proportional error in blood HA (%)	86.0 (5.9)	–
	Proportional error in blood OA (%)	73.3 (8.8)	–
	Proportional error in joint HA (%)	81.0 (8.5)	–
	Proportional error in joint OA (%)	135.0 (10.6)	–
	Additive error blood (ng/ml)	0.001 FIX	–

[a] HA indicate that the parameter estimate was based on healthy dogs

[b] OA indicate that the parameter estimate was based on dogs with OA

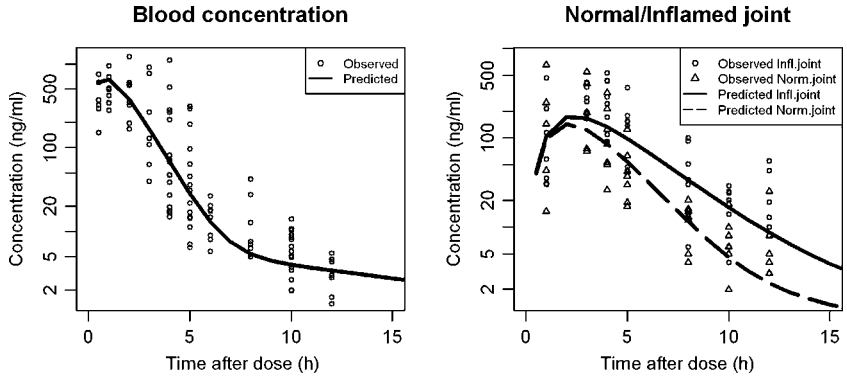

Fig. 2 Blood (*left*) and joint (*right*) robenacoxib concentrations *versus* time in the healthy dogs.

Data Evaluation

Bootstraps with 200 samples were run to assess the precision of the parameter estimates (separate bootstraps were run for the blood and the joint models). The results of the bootstraps are presented in Table I, expressed as coefficient of variation in percent (CV%). All runs that terminated either successfully or with rounding errors were included in the calculations. Overall, the parameters were found to be estimated with good precision (<30%). The high uncertainty in ka, Vp, IIV(ka) and IIV(CL/F) is, however, noticeable. The high uncertainty in the absorption parameters (ka and IIV(ka)) is probably due to the small number of individuals (n=8), as these parameters were based only on the healthy population. The high uncertainty of the peripheral volume and the IIV(CL/F) could be due to the high residual error which makes the assessment of these parameters difficult.

The VPC showed that the simulations for the healthy population were overall satisfactory, although the low number of individuals resulted in wide confidence intervals around the calculated prediction intervals (not shown). For the OA population, the data for each individual were sparser, especially for the joint, which made the performance of the VPC difficult to assess.

Shrinkage was calculated for IIV and residual variables (20). The eta-shrinkage for k_a, CL/F, MTT (mean transit time) and N (number of transit compartments) were 22%, 56%, 5% and 24%, respectively. This level of shrinkage, especially for CL/F, may limit the possibility to identify correlations between parameters. The epsilon shrinkage was only 0.6%, so it is unlikely to affect the graphical exploration significantly.

Model Predictions of Different Doses

The model was used to predict the concentration-time profiles with three different dose levels of robenacoxib: 1, 1.5 and 2 mg/kg in both healthy and OA dogs. The results are shown in Figs. 8, 9 and 10. The previously determined supposed threshold levels for COX-2 inhibition (for efficacy) and COX-1 inhibition (for safety) were included in the graph. The results showed that the time above the different threshold levels increased with increasing dose, that the concentration remained higher during a longer period of time in the inflamed joint compared to the blood, and that the OA dogs had a higher and longer exposure compared to the healthy dogs.

The predicted time above the IC_{80} for COX-2 inhibition following a 1.5 mg/kg dose in blood and inflamed joint, respectively, was approximately 3.5 and 4 h in healthy dogs compared to 5.5 and 8.5 h in the OA dog.

The predicted time above the IC_{50} for COX-2 inhibition following a 1.5 mg/kg dose in blood and inflamed joint, respectively, was approximately 6 and 10 h in healthy dogs compared to 10 and 16 h in the OA dog.

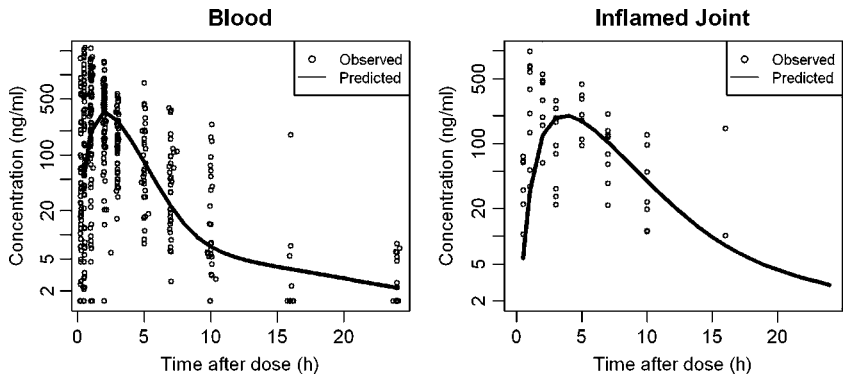

Fig. 3 Blood (*left*) and inflamed joint (*right*) robenacoxib concentrations *versus* time in the OA dogs.

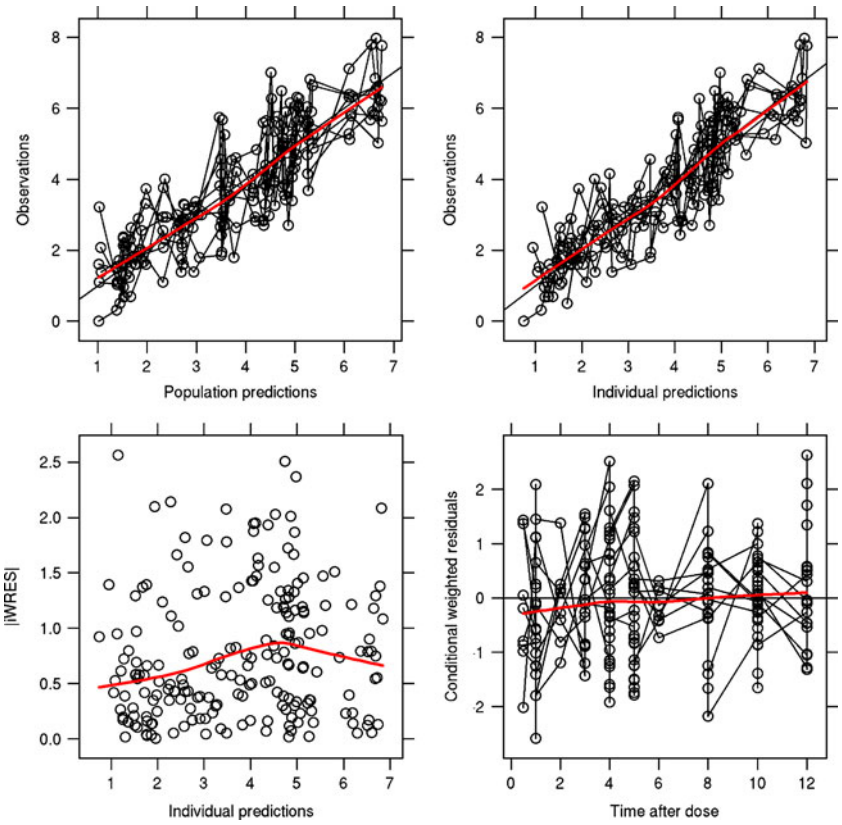

Fig. 4 Goodness-of-fit graphics for the healthy animal data. The top left graph shows the observed data *vs*. the population predictions (log-scale). The top right graph shows the observed data *vs*. individual predictions (log-scale). The line of identity is included as well as a smooth line (*in red*) through the data. The bottom left graph shows the absolute individual weighted residuals (|iWRES|) *vs*. the individual predictions, including a smooth line (*in red*) through the data. This graph was used to assess if the appropriate error model had been chosen, which is indicated by a lack of trend across the individual predictions. The bottom right graph shows the conditional weighted residuals *vs*. time after dose. The majority of the data points fall within a magnitude of +/−2 conditional weighted residuals, and there is no major trend in the data over time, indicating that there are no major model misspecifications present.

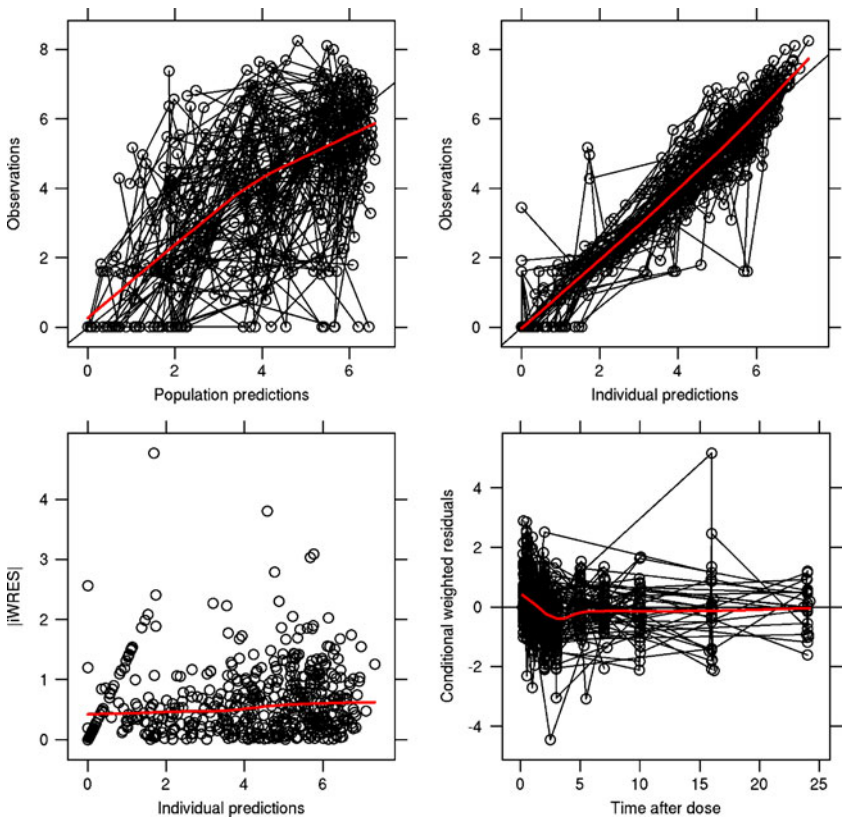

Fig. 5 Goodness-of-fit graphics for the OA animals. See Fig. 4 legend for details.

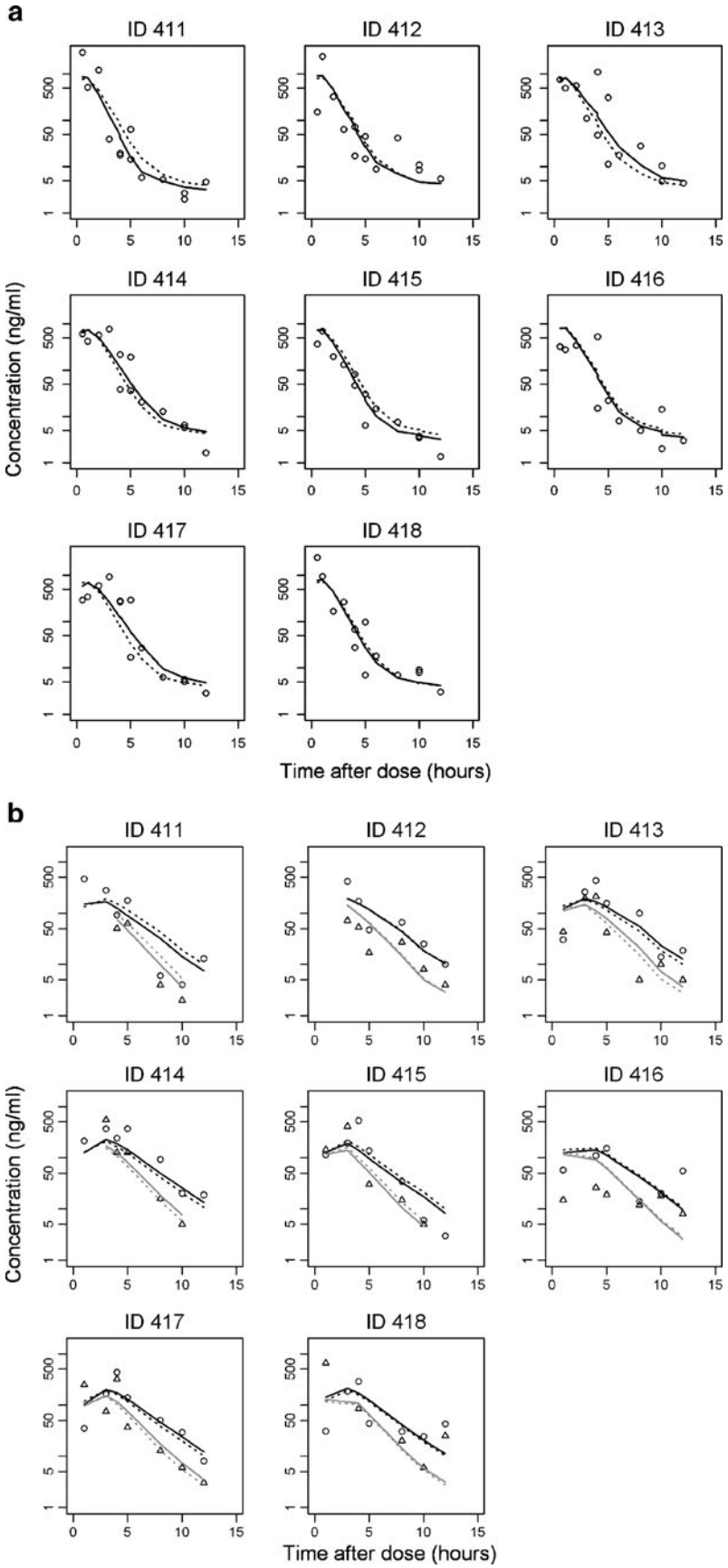

Fig. 6 **a**. Individual predictions of blood robenacoxib concentration data *versus* time for the healthy dogs. The data were collected on different occasions but have here been included in the same graph. Open circles are observed data, the dashed line is the population prediction, and the full line is the individual prediction of each individual. **b**. Individual predictions of joint robenacoxib concentration data *versus* time for the healthy dogs. The data were collected on different occasions but have here been included in the same graph. Open circles are observed data of the inflamed joint, open triangles are observed data of the normal joint, the dashed line is the population prediction, and the full line is the individual prediction of each individual. The black and the grey lines indicate the population and individual predictions of the inflamed and the normal joint, respectively.

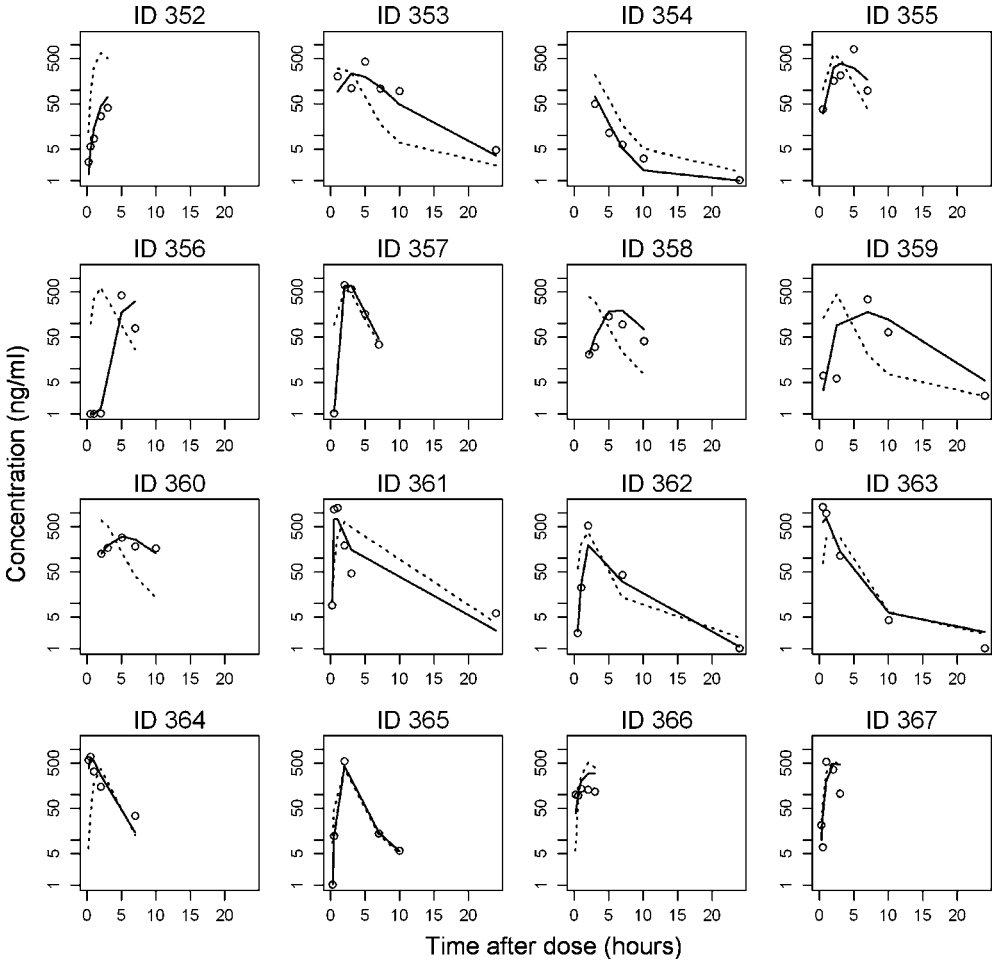

Fig. 7 Individual predictions of blood robenacoxib concentration data *versus* time of a subset of individuals from the OA dog population. Open circles are observed data, the dashed line is the population prediction, and the full line is the individual prediction of each individual.

For the non-inflamed joint in the healthy dog, the predicted time above the IC_{50} was approximately 7 h, and the predicted time above the IC_{80} approximately 2.5 h.

At all doses, including 2 mg/kg, the robenacoxib concentrations of the typical individual did not reach the IC_{20} for COX-1 inhibition in blood or inflamed synovial fluid, even at the Cmax.

DISCUSSION

The principal finding of this study is that the residence time of robenacoxib in dogs is longer in inflamed stifle joint synovial fluid compared to non-inflamed synovial fluid or blood. An additional result of clinical relevance was the fact that the clearance of robenacoxib was approximately 75% lower in dogs with OA compared to healthy Beagles. The population pharmacokinetic model selected enabled the effective analysis and comparison of the relatively sparse data obtained in the OA dogs with the slightly richer data set of the smaller population of experimental Beagles.

In human patients with rheumatoid arthritis treated with lumiracoxib, a new generation highly selective COX-2 inhibitor, high drug concentrations persisted in inflamed joints for prolonged time periods with steady-state trough concentrations approximately three times higher in synovial fluid than in plasma (11). Because the chemical structure and pharmacokinetic properties of robenacoxib closely resemble those of lumiracoxib (similarly, it contains a carboxylic acid group, is highly protein-bound, and has a low volume of distribution and a short half-life in blood), it was hypothesized that robenacoxib would also show preferential distribution and persistence at inflamed sites (12). In the studies described in the present paper, we confirmed the tissue-targeting behavior of robenacoxib in the inflamed stifle synovial fluid of dogs.

It is not possible to take frequent repeated synovial fluid samples from dogs, and therefore sparse sampling protocols followed by a population pharmacokinetic analysis were employed to estimate the population typical value for the pharmacokinetic parameters of robenacoxib as well their inter-individual variability. Because the pharmacokinetics

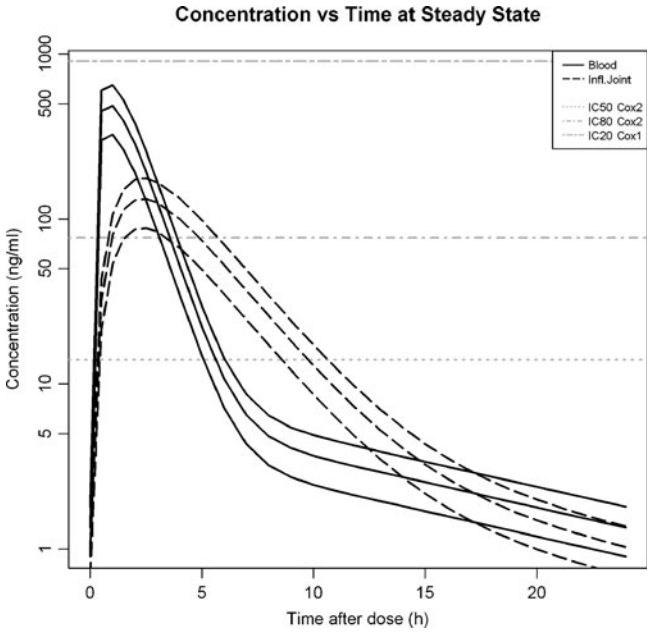

Fig. 8 Population predictions from the model showing blood and inflamed joint concentrations at steady state following a 1, 1.5 or 2 mg/kg dose in the healthy dogs. The horizontal lines indicate the IC_{50} and IC_{80} for COX-2 inhibition (efficacy) and the IC_{20} for COX-1 inhibition (safety).

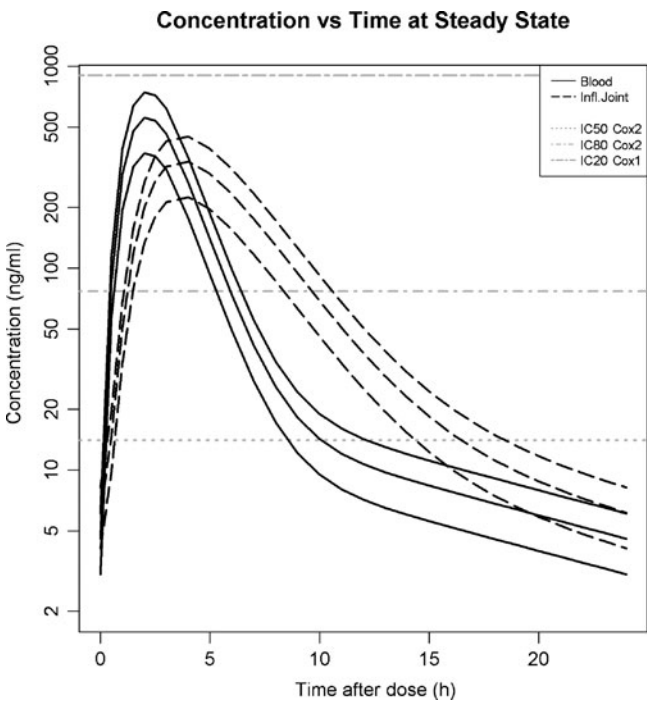

Fig. 9 Population predictions from the model showing blood and inflamed joint concentrations at steady state following a 1, 1.5 or 2 mg/kg dose in the OA dogs. The horizontal lines indicate the IC_{50} and IC_{80} for COX-2 inhibition (efficacy) and the IC_{20} for COX-1 inhibition (safety).

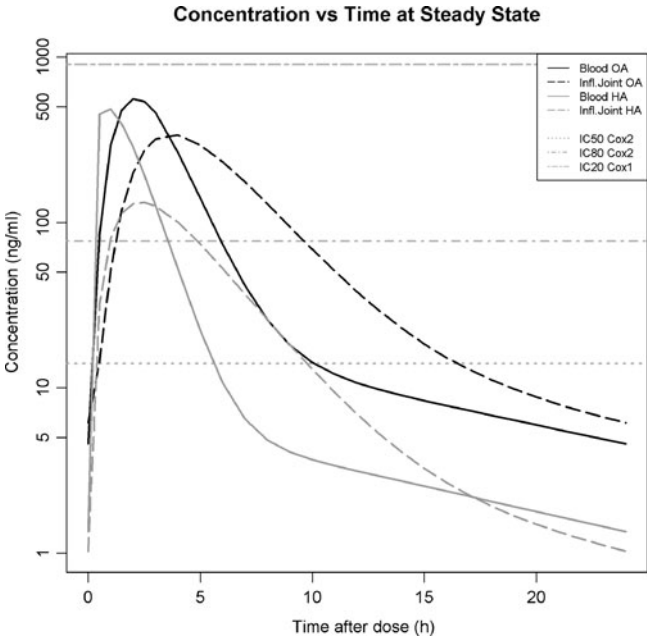

Fig. 10 Population predictions from the model showing blood and inflamed joint concentrations at steady state in healthy (HA) and OA animals following a 1.5 mg/kg dose. The grey and black curves are for healthy and OA dogs, respectively. The horizontal lines indicate the IC_{50} and IC_{80} for COX-2 inhibition (efficacy) and the IC_{20} for COX-1 inhibition (safety).

of robenacoxib are dose-linear over the range 0.5–8 mg/kg in dogs (5), it was possible to use these parameters to predict blood and inflamed synovial fluid pharmacokinetic profiles for different doses of robenacoxib over the clinical dose range of 1 to 2 mg/kg. Predictions for a dose of 1.5 mg/kg showed that robenacoxib concentrations would be above the IC_{50} for inhibition of COX-2 for approximately 16 h in the inflamed joint in the dogs with OA (*versus* 10 h in the inflamed joint of the healthy dogs), thereby providing an explanation why robenacoxib provides good efficacy with once-daily dosing for chronic OA in dogs (8). The therapeutic effect of this drug therefore persists after it has decreased to BLQ in blood, which can be attributed to sustained drug levels at the site of action (*i.e.* the effect compartment of the pharmacokinetic model). The choice of the IC_{50} for COX-2 and the IC_{20} for COX-1 as threshold concentrations for the predictions comes from the fact that it is generally considered as desirable for NSAID blood concentrations not to exceed the IC_{20} for COX-1 to ensure minimal side effects in relation to damage to the gastrointestinal tract and inhibition of hemostasis, whereas it is believed to be important to remain at least above the IC_{50} for COX-2 for efficacy (2,21).

The tissue selectivity of certain NSAIDs is attributed to their physico-chemical properties, notably the combination of being a weak acid and highly bound to proteins (12). Robenacoxib is both a weak acid (pKa 4.7) and highly protein-bound (> 98% in dogs) (4,6). This molecule will

therefore have a negative charge at the neutral pH of blood, which should limit its exit from plasma into most tissues (12). During inflammation, however, blood supply to the injured site is increased, and the endothelium becomes porous, allowing both protein-bound and unbound drug to escape into the tissues. In addition, inflammatory exudate is mildly acidic, thereby reducing protein binding and increasing the free fraction of the drug (12). As expected, the k_{out} for robenacoxib was slower in the inflamed compared to the non-inflamed joint. The slower k_{out} is explained by the ion-trapping of the molecule, the low pH of the exudate facilitating diffusion into the cells and thereby increasing intracellular drug concentrations (12). No difference was observed in the k_{out} for robenacoxib between the inflamed joints of healthy and OA dogs, even though the inflammation is more severe and shorter-lasting in the urate crystal-induced synovitis compared to OA. The finding of no observed difference is attributed to the sparse sampling protocol used for synovial fluid, which might also explain the fact that it was not possible to differentiate between k_{in} for the inflamed and for the normal joint in the healthy animals.

An additional finding of this study of clinical relevance was the result that the clearance of robenacoxib was 75% slower in dogs with OA compared to healthy Beagles. Several factors could have contributed to this result, since the Beagles were slightly older (mean 9.6 years) and much lighter (12.3 kg body weight) compared to the OA dogs (7.7 years, 33.9 kg body weight), which were of mixed breeds. Breed is unlikely to have played a major role, since pharmacokinetic parameters of robenacoxib in young and healthy dogs were similar in Beagles and mixed-breed animals (unpublished data). Therefore, the most important source of the difference in clearance may be CYP inhibition due to chronic inflammation. It is now well established that some of the underlying mediators and mechanisms involved in inflammation responses (*e.g.* cytokine production) are capable of interacting with and altering the levels and activities of, cytochrome P450 and other drug-metabolizing enzymes (22,23). Changes in drug clearance have been reported in humans and animal models associated with various inflammatory conditions ranging from surgical procedures to rheumatoid arthritis. Drug biotransformation has also been shown to be compromised as a result of CYP down-regulation in human arthritic conditions. Although there seems to be a clear difference between healthy Beagles and OA dogs in terms of rate of elimination, the clearance of robenacoxib in the target population remains rather high. This finding was expected and is a clear advantage from a drug safety point of view, because it allows several body compartments (bloodstream and blood vessels, kidney, gastrointestinal tract) to be spared from prolonged exposure to effective drug concentrations. The hypothesis of the amount of drug persisting in the inflamed joint not having an influence on the blood pharmacokinetic profile was also confirmed in the modeling process, which is further proof that the systemic exposure to robenacoxib at later post-administration time points is very likely negligible for this drug.

The absorption profile of robenacoxib was more variable in the OA dogs compared to the Beagles. This might be explained by differences in patterns of absorption in the heterogeneous clinical population compared to the homogeneous population of laboratory Beagles. The inflammation intensity and the concomitant CYP inhibition profiles could also play a role in the overall high inter-individual variability observed in the OA dogs.

A number of limitations of the modeling were identified. First, the high residual error estimated by the model indicated that there was a problem in assessing and correctly allocating the inter-individual variability to different parameters, and also in assessing the variability between occasions, which is clearly visible from graphical assessment of the raw data (not shown). The high residual error is also reflected in the rather high uncertainty seen in some parameters when performing the bootstraps.

Second, the results from the VPC partly reflected the high residual error in that the simulations created very high concentrations compared to the observed data. Because a proportional error was used, the effect of the high residual error was most significant close to the peak concentrations. This was especially obvious for the joint data and the reason why simulations were not used to predict new data. Instead, a more conservative approach was adopted in which the model was used for deterministic predictions of the typical individual rather than simulation of a new population of individuals including residual error. Only the results from the deterministic approach were used to generate the results presented here.

Third, in the healthy animals, the protocol times were used rather than actual times for sampling, which may have increased the residual error, especially during the absorption phase when concentrations often change fast.

An additional limitation is the fact that we did not measure COX inhibition directly in the blood and synovial fluid in this study, and the data used for the simulations of times above different ICs for COX inhibition were based on the results of *in vitro* whole blood assays. IC values might differ between blood and inflammatory exudate. Therefore, in future studies it is recommended to combine pharmacodynamic (*e.g.* exudate prostaglandin E_2) with pharmacokinetic measurements when sampling the site of action. Another possibility for future experimental studies would be to use microdialysis techniques to have richer data for synovial fluid and to avoid blood contamination and repeated tissue trauma that can lead to overestimation or underestimation of drug levels (24).

CONCLUSION

Robenacoxib belongs to the new generation of NSAIDs which combine high COX-2 selectivity with tissue selectivity. The tissue selectivity of robenacoxib may explain, at least partly, why robenacoxib provides good efficacy in dogs with OA or undergoing surgery when administered once daily, another possible reason being slower clearance of robenacoxib due to CYP inhibition in dogs with inflammation present. These tissue targeting properties may also explain the good safety profile of robenacoxib in dogs.

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