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Population pharmacokinetics of mavacoxib in osteoarthritic dogs

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Mavacoxib (Trocoxil™) is an oral long-acting COX-2 inhibitor approved for the treatment of osteoarthritis in dogs. Two field trials were conducted in client-owned dogs suffering from osteoarthritis, with dosages of 4 mg/kg body weight (BW) (Study 1) or 2 mg/kg BW (Study 2). Mavacoxib plasma concentrations were determined from trough blood samples and from blood samples collected at 4–10 months after the last dose. A one-compartment linear model was fitted to the concentration data (1317 concentration records from 286 patients), and parameters for oral clearance (Cl/F), apparent volume of distribution (V_d/F) and their between-subject variabilities (BSV) were estimated. Covariates were included in the model based on the outcomes of stepwise regression procedures. In the final model, the typical value of Cl/F was a function of BW, age and breed. German shepherds and Labrador retrievers were found to have 31% higher values of Cl/F than patients from different breeds with similar ages and BWs. The typical value of V_d/F was found to be dependent only on BW. The two field studies appeared to differ similarly with respect to Cl/F and V_d/F. The explanation for this difference is not known, but the difference was accounted for in the final model as a 23.9% lower bioavailability in Study 2. Mavacoxib exhibited relatively broad BSV in Cl/F and V_d/F, with coefficients of variation of 47% and 19%, respectively. The typical value for mavacoxib's terminal elimination plasma half-life ($t_{1/2}$) was 44 days, but a minority of patients (approximately 5%) had empirical Bayes estimates of $t_{1/2}$ exceeding 80 days. Simulations with the model indicated that the majority of patients treated with mavacoxib 2 mg/kg will maintain trough plasma mavacoxib concentrations associated with efficacy. Results of the population pharmacokinetic analysis helped to reduce the dose from 4 to 2 mg/kg and thus increased the therapeutic index for this molecule.

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INTRODUCTION

Mavacoxib is a nonsteroidal anti-inflammatory drug and is a member of the coxib class of selective cyclooxygenase-2 (COX-2) inhibitors. Mavacoxib (Trocoxil™) is approved for the treatment of pain and inflammation associated with degenerative joint disease in dogs in cases where continuous treatment exceeding one month is indicated (EMEA, 2008). The pharmacokinetics of mavacoxib in young adult laboratory Beagle dogs has been described recently (Cox *et al.*, 2010). Mavacoxib is characterized by an extremely low clearance of 2.7 mL/h/kg, a relatively large apparent volume of distribution at steady-state of 1.6 L/kg, and a prolonged plasma $t_{1/2}$ ranging from 8 to 39 days, with a median of approximately 17 days. When mavacoxib tablets were administered at a dose of 4 mg/kg body weight (BW) to fasted Beagle dogs, the absolute bioavailability (F) of mavacoxib was 46.1%; administration with food increased the bioavailability to 87.4%. Dose-ranging studies in laboratory models of inflammation and canine whole blood assays for COX-2 activity indicated that efficacy would be provided by a minimum target trough plasma mavacoxib concentration of approximately 0.4 μg/mL (Lees *et al.*, 2009). In order to achieve steady-state concentrations rapidly with a molecule with such an extended plasma elimination half-life, mavacoxib is administered with a regimen involving a 2-week interval between the first and second doses but with monthly dosing thereafter.

This study describes the population pharmacokinetic data from two field trials with mavacoxib in osteoarthritic patients that tended to be elderly large-breed dogs. The safety and efficacy data from these studies are described elsewhere (Payne-Johnson *et al.*, 2009). Before the field trials were performed, a nonlinear mixed effects model was developed to describe mavacoxib pharmacokinetics in Beagle dogs, based on data from several studies with intensive blood sampling. The structural pharmacokinetic model that adequately described the beagle data was an open one-compartment linear disposition model with first-order oral absorption. Simulations with the model indicated the adequacy of the trough blood-sampling schemes used in the field trials to characterize the population pharmacokinetics of mavacoxib in osteoarthritic patients provided that trough samples were obtained, at minimum, after doses 1, 2 and 4. The simulations also indicated that the estimates of the population pharmacokinetic parameters (e.g. oral clearance and apparent volume of distribution) would be negligibly biased if the oral absorption step were ignored, i.e. an adequate base structural model for the trough concentrations from osteoarthritic patients could be a simpler structural model with bolus administration. More information about the Beagle dog mixed-effect model is presented in the Supporting Information.

MATERIALS AND METHODS

Osteoarthritic patient studies

Clinical study design.

Two similar multicenter randomized, parallel group studies with a positive control were conducted in accordance with VICH GCP standards (VICH, 2000; Payne-Johnson *et al.*, 2009). Osteoarthritic dogs enrolled in the studies were randomized to receive treatment with mavacoxib and daily placebo for carprofen (Rimadyl™ Chewable Tablets; Pfizer Animal Health, New York, NY, USA) or placebo for mavacoxib and daily carprofen at a nominal dose of 4 mg/kg BW. Mavacoxib was administered in both studies with a 2-week interval between the first and second doses but with monthly dosing thereafter. The nominal mavacoxib doses in Studies 1 and 2 were 4 and 2 mg/kg BW, respectively. Seven mavacoxib doses were administered in Study 1, but only five doses in Study 2. In Study 1, mavacoxib was administered without regard to the timing of meals, but in Study 2, all of the mavacoxib doses were administered with food.

Pretreatment and trough blood samples were collected in both studies for the measurement of plasma mavacoxib concentrations. In Study 1, the trough blood samples were collected after mavacoxib doses 1, 2, 4, 6 and 7. In Study 2, the trough blood samples were collected after each dose. Following a prolonged washout period of several months after completion of the drug administration phase of the studies, additional single blood samples were collected from many patients for the determination of residual plasma mavacoxib concentrations. For Study 1, the additional late blood samples were collected from 145 of 224 patients (65%) treated with mavacoxib at times ranging from 162 to 310 days after their last mavacoxib doses. For Study 2, the late blood samples were collected from 52 of 62 patients (84%) at 116–193 days after their last mavacoxib doses. These late samples were used to confirm the prolonged plasma $t_{1/2}$ of mavacoxib in osteoarthritic patients.

Analytical methodology

For all of the studies, the plasma concentrations of mavacoxib were determined by one laboratory using an LC/MS/MS procedure with a lower limit of quantification (LLOQ) of 5 ng/mL, a lower limit of detection of 1.6 ng/mL and a precision <5.1% (Cox *et al.*, 2010).

Dataset preparation and patient demographics

In addition to both dose and plasma concentration records, the dataset also included various patient demographic variables, e.g. sex category, breed category, age (years), BW (kg), concomitant medication category and baseline values of various laboratory tests. Purebred dogs were categorized according to breed if the dataset contained at least four patients in the breed. Concomitant medications were classified by pharmacologic category if there were at least four patients with a particular class of medications. Given the prolonged plasma elimination half-life of mavacoxib and the likelihood that a prolonged effect of a medication would be required for a noticeable effect on mavacoxib pharmacokinetics, only concomitant medications taken for at least 7 days were included in the dataset. Patient demographic data from the studies are summarized in Table 1. All postabsorptive phase plasma samples (i.e. samples obtained at least 7 days after drug administration) with available date and time of sampling and time of latest dose administration were used for the population pharmacokinetic analysis, even if the concentrations were estimated to be below the limit of quantification (BLQ) of the assay. The disposition of all of the concentration records is listed in Table 2, and trough and late sample concentration records are summarized in Table 3.

Modeling software and methodology

The nonlinear mixed effect modeling was performed with the program NONMEM v. 6.1.0. (ICON Development Solutions, Ellicott City, MD, USA). Wings for NONMEM (http://wfn.sourceforge.net/index.html) was used with the patient data to organize NONMEM runs and to perform model validation using the bootstrapping. The base structural pharmacokinetic model was an open one-compartment linear pharmacokinetic model with bolus input. An exponential error model was used to describe the between-subject variability (BSV) in pharmacokinetic parameters Cl/F and V_d/F:

$$Cl/F_j = \theta(Cl/F) \times \exp(\eta_j(Cl/F)), \quad (1)$$

$$V_d/F_j = \theta(V_d/F) \times \exp(\eta_j(V_d/F)). \quad (2)$$

In the above equations, Cl/F_j and V_d/F_j are the apparent clearance and volume of distribution, respectively, for the jth

Table 1. Summary of demographics for patients in the dataset

Variable	Statistic or category	Study 1	Study 2	All
Age (years)	*N*	224	62	286
	Mean (SD)	9.3 (3.1)	10.1 (3.0)	9.5 (3.1)
	Median	10.0	11.0	10.0
	Minimum–maximum	1–18	1–15	1–18
Sex, *N* (%)	*N*	224	62	286
	Male, Intact	86 (38.4)	32 (51.6)	118 (41.3)
	Female, Intact	28 (12.5)	30 (48.4)	58 (20.3)
	Female, Neuter	72 (32.1)	0 (0.0)	72 (25.2)
	Male, Neuter	38 (17.0)	0 (0.0)	38 (13.3)
Breed, *N* (%)	*N*	224	62	286
	German Shepherd	28 (12.5)	8 (12.9)	36 (12.6)
	Labrador Retriever	30 (13.4)	11 (17.7)	41 (14.3)
	Golden Retriever	15 (6.7)	1 (1.6)	16 (5.6)
	Others*	151 (67.4)	42 (67.6)	193 (67.5)
Weight (kg)	*N*	224	62	286
	Mean (SD)	34.0 (11.6)	30.6 (12.4)	33.3 (11.8)
	Median	34.7	32.1	34.3
	Minimum–maximum	4.6–67.0	4.8–65.0	4.6–67.0

*Includes four to seven purebred patients of each of the following breeds: Collie, English Setter, Brittany Spaniel, Newfoundland, Boxer, Beauceron, Rottweiler and Burmese Mountain dog.

Table 2. Disposition of mavacoxib concentration samples in the dataset

	Study		
Summary variable	Study 1	Study 2	All
Number of dogs providing PK samples	224	62	286
Total number of PK samples	957	362	1319
Number of outlier samples excluded from analysis	0	0	0
Number of samples excluded for other reasons	2*	0	2*
Number of samples with assay results BLQ	40	7	47
Total number of samples included in the final dataset	955	362	1317

*Blood samples were collected 1 day after dosing and could not be considered to be in the terminal disposition phase.

subject, and $\theta(Cl/F)$ and $\theta(V_d/F)$ represent the typical values of Cl/F and V_d/F for the population. In the base model, the $\theta(Cl/F)$ and $\theta(V_d/F)$ are the typical values, unadjusted for any covariates. In the full model, the $\theta(Cl/F)$ and $\theta(V_d/F)$ may be functions of explanatory covariates (*vide infra*). Additionally, the η variables are random and normally distributed with mean 0 and variances $\omega^2(Cl/F)$ and $\omega^2(V_d/F)$, respectively, and represent the BSV in Cl/F and V_d/F. Plasma concentrations of mavacoxib for each subject were predicted as a function of time from the model parameters, and residual variability in the concentrations was modeled as a sum of proportional and additive errors. Equation (3) shows the relationship between the observed and predicted concentrations.

$$C_{ij} = \hat{C}_{ij} \times (1 + \varepsilon 1_{ij}) + \varepsilon 2_{ij}. \quad (3)$$

In the above equation, C_{ij} is the ith measured plasma concentration in the jth subject, and $\hat{C}_{ij}$ is the ith model-predicted concentration in the jth subject. In addition, $\epsilon 1_{ij}$ and $\epsilon 2_{ij}$ are normally distributed random variables with mean 0 and with the variance of the $\epsilon 1_{ij}$ given by $\sigma 1^2$. The additive error term was used to stabilize the model, with its standard deviation fixed at half of the LLOQ. A small proportion (3.6%,) of the samples in the entire patient dataset were BLQ, and most of these samples were the late samples in Study 1, when the median time of collection was approximately 8 months after the last mavacoxib dose. A conditional likelihood estimation method, the F_FLAG method, was used with the BLQ samples (Bergstrand *et al.*, 2007).

The contributions of the various discrete and continuous variables in the dataset were evaluated on the model's ability to describe the observed concentration data through a series of forward selection/backward elimination regressions. In the forward selection regressions, the effect of each potential explanatory variable was evaluated individually against the base model and found to significantly improve the model if the objective function was reduced by more than 3.84 (nominal $P < 0.05$ in the likelihood ratio test). The association of study with bioavailability was also similarly evaluated in the model building exercise. The following examples show how continuous and discrete variables were added to the population pharmacokinetic model.

$$\text{Continuous variable:} \quad Cl/F = \theta 1 \times (\text{weight/median weight})^{\theta 2}, \quad (4)$$

$$\text{Discrete variable:} \quad Cl/F = \theta 1 \times (1 + \theta \times \text{sex}). \quad (5)$$

In Equation (5), sex is an indicator variable with a value of 0 for male and 1 for female patients. The $\theta 1$ values in Equations (4) and (5) are the typical values of Cl/F with median BW or for males, respectively, and the BW normalization in Equation (4) was used to stabilize the model (Bonate, 2006). All of the explanatory variables that met the criterion in the model

Table 3. Summary of plasma mavacoxib concentration data stratified by study and number of doses

Study	Sample ID	N	Mean ± SD (μg/mL)	Minimum–maximum (μg/mL)
1	Postdose 1 (study day 14)	210	1.39 ± 0.40	BLQ*–2.45
	Postdose 2 (study day 45)	203	1.91 ± 0.74	BLQ–3.84
	Postdose 4 (study day 105)	185	2.45 ± 1.19	0.006–7.55
	Postdose 6 (study day 165	65	2.58 ± 1.31	0.53–6.68
	Postdose 7 (study day 195)	98	2.60 ± 1.58	BLQ–8.75
	Late Sample (162–310 days after last dose, median = 246 days)	145	0.045†	BLQ–2.94
2	Postdose 1 (study day 14)	61	0.52 ± 0.15	BLQ–0.82
	Postdose 2 (study day 45)	62	0.73 ± 0.30	0.03–1.34
	Postdose 3 (study day 75)	60	0.88 ± 0.41	0.08–1.74
	Postdose 4 (study day 105	58	0.97 ± 0.47	0.12–1.76
	Postdose 5 (study day 135)	56	1.11 ± 0.50	0.10–2.36
	Late sample (116–193 days after last dose, median = 169 days)	52	0.137†	BLQ–0.79

For simplicity in evaluating trough concentrations after each dose, only samples collected within 11–18 days after the first dose and 24–35 days after subsequent doses were included in the calculations presented in this table.

*Below the limit of quantification (BLQ), 0.005 μg/mL.

†Median with no calculation of SD.

building step were included into the full model. If the effects of two explanatory variables were apparently highly correlated, the variable with the largest decrease in the objective function from the base model was used. The contribution of each explanatory variable was then assessed in a series of stepwise regressions in which the explanatory variables were removed from the full model and the increase in the objective function was evaluated. The explanatory variables were considered to contribute significantly to the full model if the objective function increased by more than 7.88 (nominal $P < 0.005$ in likelihood ratio test) when the variable was removed from the model. The final model was selected when all of the explanatory variables contributed significantly to the model's ability to describe the data.

Bayesian *post hoc* estimates were obtained with the base and final models, and traditional graphical presentations were created to evaluate goodness of fit (Jonsson *et al.*, 2007). Shrinkage of random effects toward the means could have occurred due to the sparse pharmacokinetic sampling, and a metric for shrinkage[1] was calculated for the ηs and ϵ (Karlsson & Savic, 2007). The final model was tested for stability by the bootstrap technique (Parke *et al.*, 1999). Simulations with the final model were also performed by using NONMEM in the simulation mode. In the simulations, mavacoxib trough concentrations were generated for at least 5000 patients treated according to the protocol-specified procedures. Descriptive statistics were calculated for the concentrations and compared with a 0.4-μg/mL concentration associated with efficacy in laboratory models of inflammation (Lees *et al.*, 2009).

[1]Shrinkage of ηs was calculated as $1 - \mathrm{SD(EBEs)}/\omega$, where SD(EBEs) is the standard deviation of the empirical Bayes estimates of the ηs, and ω is the population model estimate of the standard deviation of the η. Shrinkage of ϵ was calculated as $1 - \mathrm{SD(IWRES)}$, where SD(IWRES) is the standard deviation of the individual weighted residuals.

RESULTS

Initial model and pharmacokinetic differences in studies

There were 19 concentrations (1.4%) with values of population-predicted weighted residuals > 5 in the final population pharmacokinetic model. For each of these potential outliers, the samples were collected at more than 150 days after the last dose and the observed mavacoxib concentrations were higher than the population-predicted concentrations.

Pharmacokinetic data from the 4-mg/kg field study (Study 1) were available before the 2 mg/kg BW field study (Study 2) was begun. A population pharmacokinetic model for mavacoxib was developed from these initial data, and the data from Study 2 were first used as an external validation dataset for the model. In the validation exercise, the distribution of between-subject effects (i.e. the ηs) on Cl/F and V_d/F in Study 2 were not symmetrically distributed around zero, and the median empirical Bayesian estimates of Cl/F and V_d/F in the study were greater than the model parameter values from Study 1 by 24.4% and 13.8%, respectively. Although the reason for these similar differences in Cl/F and V_d/F from the model parameter values is not known, the differences could be explained by lower bioavailability (F) of mavacoxib in Study 2 and indicated the need for model refinement. Data from Studies 1 and 2 were then pooled and renewed model development work began, starting with the base model.

Base model

With the exception of a few outliers, the base model adequately described the mavacoxib plasma concentrations in most patients from the two studies. The typical values and percent relative standard errors of Cl/F and V_d/F were 1.42 (4.4%) L/day and

79.2 (3.6%) L, respectively. The typical $t_{1/2}$, based on these values, was 38.1 days. The BSVs in Cl/F and V_d/F were large in the base model (65.4% and 52.3% coefficients of variation, respectively), but the estimates of Bayesian shrinkage of the random effects of Cl/F and V_d/F were small, 2.2% and 6.0%, respectively, and provided support for the general reliability of the estimates of the random effects. Shrinkage of ϵ was moderate, at 16%, but below levels associated with problems in the use of diagnostic plots with the individual predictions (Karlsson & Savic, 2007).

Model building

Body weight was found to be the primary factor predicting mavacoxib Cl/F and V_d/F and resulted in a reduction in the minimum value of the objective function by 353.8 units relative to the base model with no covariates. The addition of BW to the model also reduced the BSV of Cl/F from 65.4% to 49.3%, a 25% reduction, and the BSV in V_d/F was reduced from 52.3% to 23.6%, a 55% reduction. Because of the profound effect of BW in the model, this model was then used as the reference model in subsequent model-building steps. In subsequent model-building work, the full model included the effects of study on F, BW on V_d/F, and BW, age, serum alkaline phosphatase (ALKP), sex and breed on Cl/F.[2] The effect of breed on Cl/F only tested the potential effect of German shepherds and Labrador retrievers on Cl/F; other breeds were not tested because exploratory graphical analysis indicated no meaningful effects of the other pure breeds in the patient dataset. The stepwise reductions eliminated serum ALKP and sex from effects on Cl/F, leaving the final model with effects of study on F, BW on V_d/F, and BW, age and breed on Cl/F.

[2]In the full model, the typical values of Cl/F (TVCL), V_d/F (TVVd) and relative bioavailability (TVF) were coded as the following:

$$\mathrm{TVCL} = \theta_{Cl}(\mathrm{BW}/35)^{\theta Cl\_\mathrm{BW}}(\mathrm{Age}/10)^{\theta\mathrm{Age}}(\mathrm{ALP}/35)^{\theta\mathrm{ALP}}(1+\mathrm{SEX}\theta_{\mathrm{SEX}})(1+\mathrm{BREED}\theta_{\mathrm{BREED}}),$$

$$\mathrm{TVVd} = \theta_{V_d}(\mathrm{BW}/35)^{\theta V_d\_\mathrm{BW}},$$

$$\mathrm{TVF} = 1+\mathrm{STD}\theta_{\mathrm{STUDY}},$$

where BW is patients' BW in kg, Age is patients' age in years, ALP is the value for laboratory test ALKP (U/L), θ_{Cl_BW}, θ_{AGE} and θ_{ALKP} are allometric coefficients of covariate functions on Cl/F for BW, age and ALKP, respectively. The $\theta_{V_d\_BW}$ is the allometric coefficient for BW on V_d/F. SEX is an indicator variable with values of 0 for males and 1 for females, BREED is an indicator variable for patient breed with values of 1 for German shepherds and Labrador retrievers but 0 otherwise, and STD is an indicator variable with value of 0 for Study 1 but 1 for Study 2. The θ_{CL} is the typical value of Cl/F for male patients who have median BW, median age, median value of serum ALKP and are breeds other than German shepherds or Labrador retrievers. The θ_{Vd} is the typical value of V_d/F for patients with median BW. The denominators of the power functions for BW, age and ALKP were the median values for the covariates. In NONMEM, relative bioavailability is coded separately from either Cl/F or V_d/F, and the typical value of relative bioavailability in Study 1 was arbitrarily coded as 1 and θ_{STUDY} is the relative change in bioavailability in Study 2.

Table 4. Parameter values for the final model

Parameter or effect	Mean*	BSV†*
Cl/F (L/day)‡,§	1.35 (1.26–1.50)	46.9% (41.8–51.7%)
V_d/F (L)‡,¶	85.7 (83.0–90.3)	19.4% (12.9–24.6%)
Effect of WT on Cl/F §	0.787 (0.681–0.947)	–
Effect of WT on V_d/F ¶	0.981 (0.916–1.06)	–
Effect of age on Cl/F §	−0.215 ((−0.326)–(−0.187))	–
Effect of breed on Cl/F §	0.314 (0.176–0.559)	–
Effect of study on F**	−0.239 ((−0.291)–(0.187))	–
Half-life†† (d)	44.0	–

Proportional residual error 22.3%.

*Parameter precision is expressed as the 95% confidence interval from the bootstrap simulations.

†Between-subject variability (BSV) calculated as $(\text{variance})^{1/2} \times 100\%$.

‡Correlation between CL/F and Vd/F is 0.09, calculated as $\text{covariance}_{12} \div (\text{variance}_1 * \text{variance}_2)^{1/2}$, where variance_1 and variance_2 are variances of random effects for the two parameters and covariance_{12} is their covariance.

§Cl/F (L/day) $= 1.35 \times (\mathrm{WT}/35)^{0.787} \times (\mathrm{Age}/10)^{-0.215} \times (1 + 0.314 \times \mathrm{Breed})$ L/day, where Breed is an indicator variable with a value of 1 for Labrador retrievers or German shepherds, but 0 otherwise.

¶V_d/F (L) $= 85.7 \times (\mathrm{WT}/35)^{0.981}$.

**$F = 1.0$ for Study 1 and 0.761 for Study 2 (23.9% lower than in Study 1).

††Half-life $= 0.693 \times V_d/F \div Cl/F$, and the reported $t_{1/2}$ is for a 10-year-old 35 kg patient that is not a Labrador retriever or German shepherd.

Final model

Parameter estimates for the final model are listed in Table 4. In the final model, the bioavailability of mavacoxib was lower in Study 2 by 23.9%, with a 95% confidence interval of 18.7–29.1%. Relative to the model with BW as the only covariate, the addition of the other covariates and explanatory variables reduced the BSV of Cl/F only slightly from 49.3% to 46.9%, and the BSV of V_d/F was reduced from 23.6% to 19.4%. The basic goodness-of-fit diagnostic plots for the final population pharmacokinetic model are shown in Fig. 1. In Fig. 1c,d, there is an apparent trend for positive weighted residuals, i.e. under prediction of concentrations, at the late time points and low concentrations after discontinuation of treatment with mavacoxib. Almost all of the BLQ samples occurred at these late time points, however, and weighted residuals for the BLQ samples were not computed by NONMEM when it entered into conditional likelihood estimation. If weighted residuals could have been computed for these BLQ samples, the vast majority of them would have been negative and the apparent imbalance of positive and negative weighted residuals at these late time points and low concentrations would have been substantially corrected.

The $t_{1/2}$ values had a right-skewed distribution (Fig. 2), and 13 of the 286 patients (4.6%) had a prolonged mavacoxib $t_{1/2}$, with values ranging from 80 to 140 days. The observed and model predicted concentrations for two representative patients with half-lives >80 days are presented in Fig. 3. As shown in this figure, the trough concentrations of some patients continued

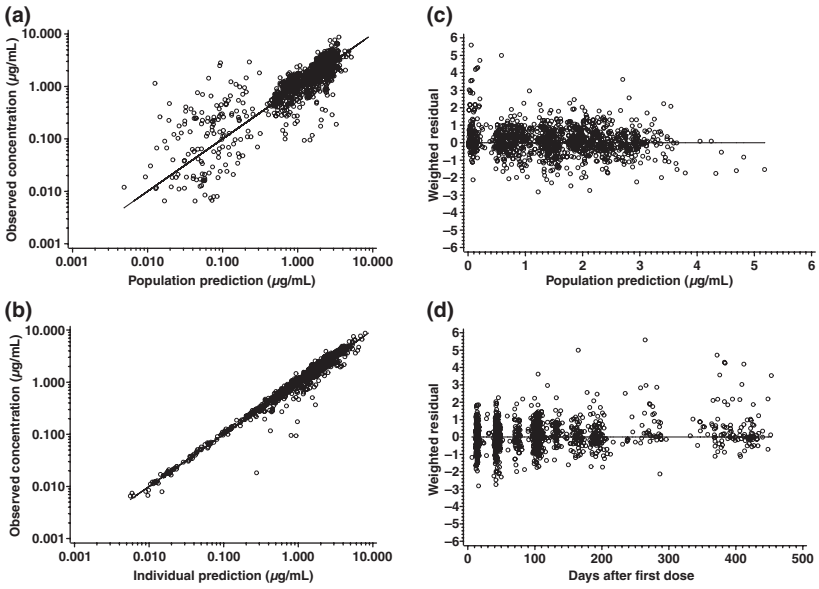

Fig. 1. Goodness-of-fit plots for the final model. (a) Population predicted concentrations vs. observed concentrations, with line of unity. (b) Individual predicted concentrations vs. observed concentrations, with line of unity. (c) Weighted residuals vs. population predicted concentrations. (d) Weighted residuals vs. time.

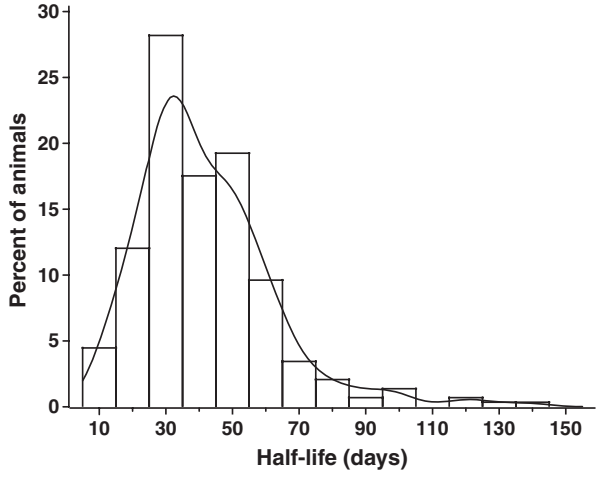

Fig. 2. Histogram of the empirical Bayes estimates for $t_{1/2}$ (n = 286 patients). The solid line is the kernel density function, an empirical estimate of the distribution of $t_{1/2}$ values.

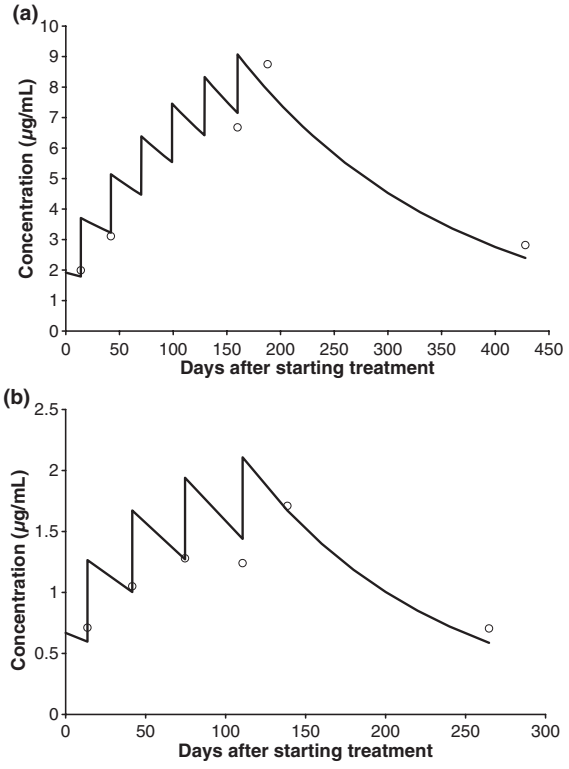

Fig. 3. Representative mavacoxib concentration data from patients with $t_{1/2}$ values longer than 80 days. Patients A (a) and B (b) received nominal mavacoxib doses of 4 and 2 mg/kg BW, respectively. Patient A had the highest trough and late sample concentrations in Study 1. Key: circles = observed trough and late concentrations, lines = individual predicted concentration–time profiles. Note the different scales on the x- and y-axes of the graphs.

to rise throughout the drug administration phases of the studies. The incidence of prolonged $t_{1/2}$ appeared to be similar for both the 2 and 4 mg/kg BW doses [11/286 patients in Study 1 (4.9%) and 2/62 patient in Study 2 (3.2%)] and there was no obvious association of any covariate factor with prolonged $t_{1/2}$. The patients with prolonged $t_{1/2}$ were represented by a diverse variety of breeds, ranged in BW from 4.7 to 63.2 kg (median 34 kg), were six females and seven males, and ranged in age from 6 to 14 years (median = 11 years). (See the section on Covariate effects.)

There were 19 concentrations (1.4%) with values of population-predicted weighted residuals >5 in the final PPK model. For each of these potential outliers, the samples were collected at more than 150 days after the last dose and the observed mavacoxib concentrations were higher than the population-predicted concentrations. The outliers all came from different patients and 9 of the 19 dogs had $t_{1/2}$ estimates

>80 days. The large residual errors in these predicted concentrations may be expected because the long time intervals (i.e. multiple $t_{1/2}$ values after the last dose) would amplify any biases in the predicted estimates of *Cl/F* and V_d/F. When the outliers were excluded from the dataset, no model parameter changed by more than 8.4% from the fit with all of the data. Given the importance of the late samples in estimating $t_{1/2}$ (see Model validation section) and because of these relatively small changes in parameter estimates, the outliers were retained in the dataset.

Covariate effects

Body weight was the most important covariate factor affecting *Cl/F* and V_d/F of mavacoxib. The model-predicted values of *Cl/F* and V_d/F were power functions of BW with coefficients of 0.787 and 0.981, respectively (Table 4). The typical values of BW normalized *Cl/F* and V_d/F were 0.039 L/day/kg (1.35 L/day ÷ 35 kg median BW) and 2.45 L/kg (85.7 L ÷ 35 kg median BW), respectively. Dosage adjustment of mavacoxib based on the BW of the patients is predicted to significantly minimize the variability in drug exposure during treatment. Plots for the effect of BW on model parameters are shown in Fig. 4. The model-predicted *Cl/F* and V_d/F of mavacoxib increased with BW, and $t_{1/2}$ was also predicted to increase slightly with BW.

Most of the dogs in this population were elderly, with the median age of 10 years and with <7% of dogs having an age younger than 5 years. Age was a significant factor affecting mavacoxib *Cl/F*, but not V_d/F. The model-predicted *Cl/F* was related to a power function of age with a coefficient of −0.215, i.e. the younger the patient, the higher the *Cl/F* of mavacoxib. The model-predicted *Cl/F* is 1.35 L/day for a 35 kg patient with the median age of 10 years, 1.57 L/day for a 5-year-old 35 kg patient $[1.35 \times (5/10)]^{-0.215}$, which is about 16% higher than that of the 10-year-old patient. Breed was also a significant covariate in the final model, with a mavacoxib *Cl/F* of 1.77 L/day (~30% higher) in German shepherds and Labrador retrievers than in other patients of comparable age and BW. Overall, the effects of breed and age accounted for a small proportion of the BSV in *Cl/F*.

Model validation

The late blood samples were used to confirm the empirical Bayesian estimates of plasma elimination half-life with special emphasis on the 13 patients (4.6%) exhibiting a plasma elimination half-life of longer than 80 days. Of the 197 patients with late samples in Studies 1 and 2, a trough concentration was collected after the last mavacoxib dose for 111 of the patients. The 'observed' half-life for these 111 patients was estimated from the log-linear slope of a straight line connecting the two concentrations after the last dose. The empirical Bayesian estimates of individual half-lives from the final population pharmacokinetic model were in good agreement with the 'observed' half-lives for these patients, as shown in Fig. 5. When the late blood samples were excluded from the dataset, however, the empirical Bayesian estimates of individual half-lives were under-estimated in many of the patients. Such a finding is consistent with Bayesian shrinkage in the absence of late blood sampling and emphasizes the importance of these late blood samples in identifying patients in the upper fringe of the distribution of plasma elimination half-life. Late blood samples were collected from 69% of the patients (197 of 286), so it is possible that additional patients would have been identified as

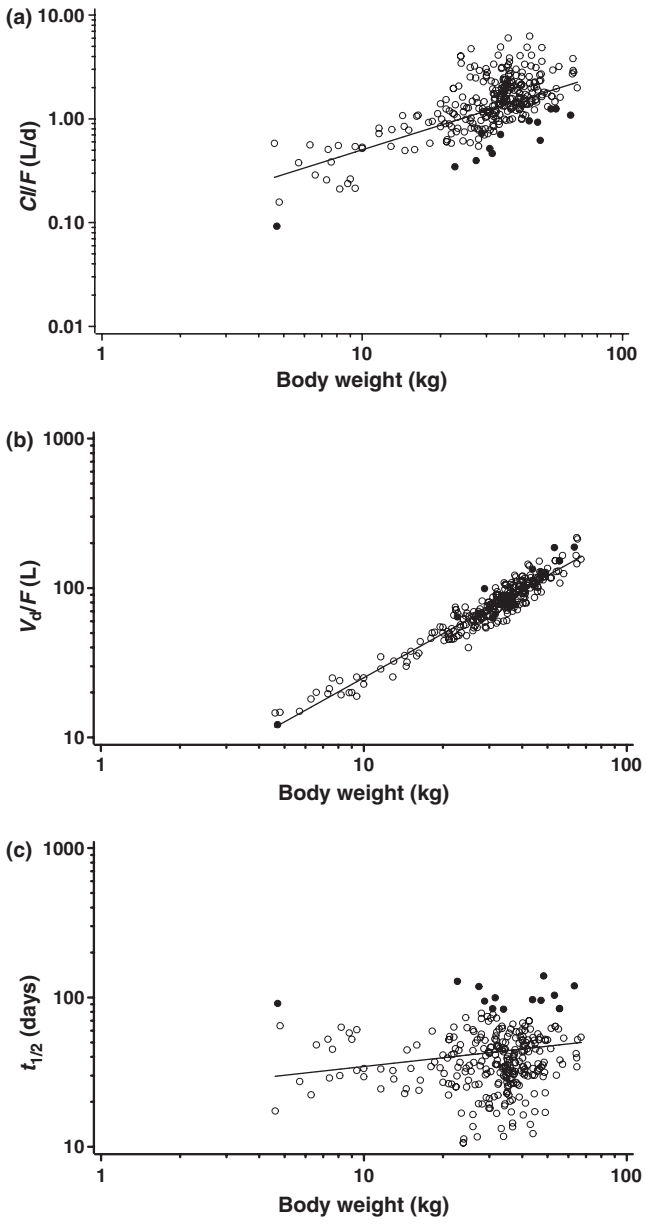

Fig. 4. Dependency of *Cl/F* (a), V_d/F (b) and $t_{1/2}$ (c) on BW. Key to symbols: the closed circles represent patients with $t_{1/2}$ > 80 days, and the open circles represent the other patients. The solid lines in are the model predicted typical values based on BW. *Cl/F* and $t_{1/2}$ are also functions of age and breed, and the solid lines in (a) and (c) are the model predicted values as a function of BW for patients that are not German shepherds or Labrador retrievers and have median age.

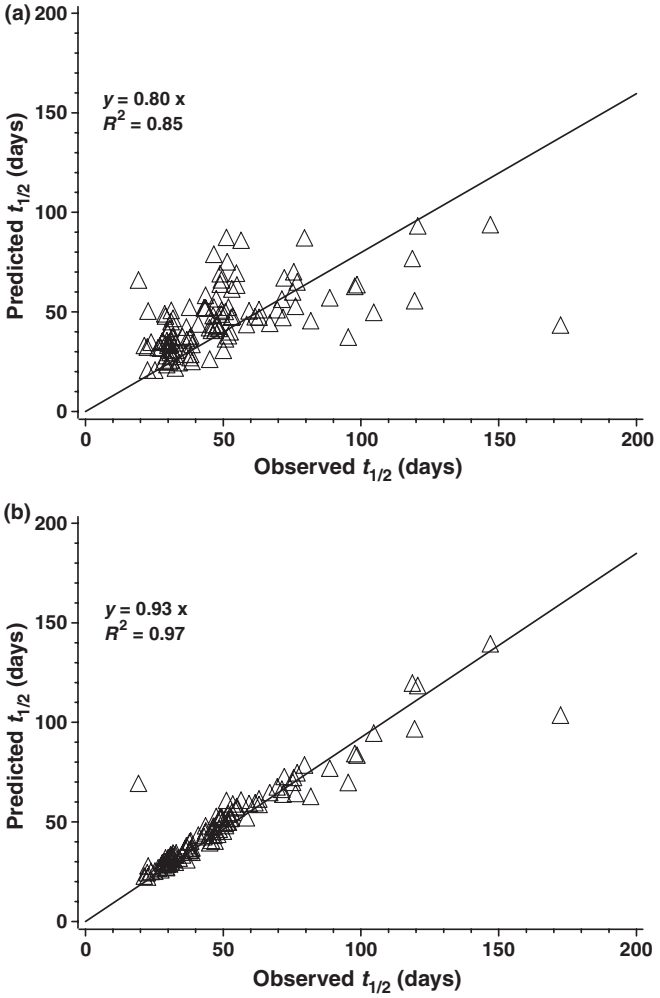

Fig. 5. Comparison of observed and predicted $t_{1/2}$ values of mavacoxib when the $t_{1/2}$ was estimated without (a) and with (b) the samples from the study extensions (n = 111).

having prolonged $t_{1/2}$ if late blood samples had been collected from all of the patients.

The final model was tested for stability by bootstrapping. The mean estimates from the 1000 bootstrap datasets were in good agreement with final population pharmacokinetic model parameters; the bootstrap mean values differed from the population pharmacokinetic model estimates by <6% except for a 11.1% difference for the effect of age on *Cl/F*. The bootstrap standard errors of the parameters tended to be larger than the model estimates, however, and the 95% confidence intervals in Table 4 are derived from bootstrapping. The generally good agreement between the population pharmacokinetic parameter estimates and the bootstrap estimates demonstrates the good precision and stability of the final model.

Simulations

Simulated trough plasma mavacoxib concentrations for >10 000 patients matching the demographics of the patients in the two studies are shown in Fig. 6. This simulation indicates that the majority of patients (≥85%) treated with the nominal 2 mg/kg BW dose should achieve therapeutic concentrations, i.e. trough concentrations ≥0.4 μg/mL, and that, on average, steady-state concentrations will be achieved by approximately 3–4 months. Few patients are predicted to have trough plasma mavacoxib concentrations exceeding 3 μg/mL.

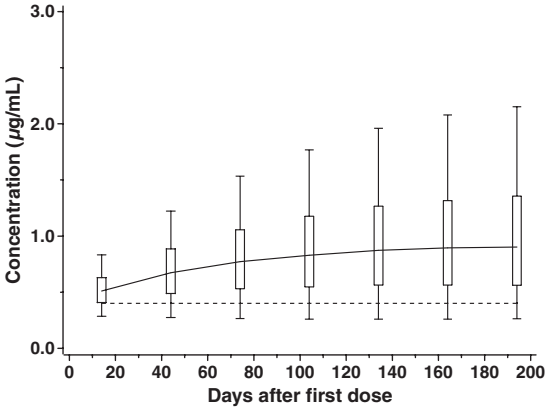

Fig. 6. Simulated trough plasma mavacoxib concentrations for patients receiving mavacoxib 2 mg/kg BW. Lines connect median values, the boxes represent the 25th–75th percentiles, and the whiskers represent the 5th–95th percentiles. The horizontal dashed line is 0.4 μg/mL.

DISCUSSION

To the authors' knowledge, this is the first use of population pharmacokinetic modeling of data from a field trial to support the registration of a veterinary medicine, in this case a unique, long-acting COX-2 inhibitor for the treatment of osteoarthritis in dogs. The population pharmacokinetic program for mavacoxib was undertaken, in part, because the preclinical laboratory pharmacokinetic, pharmacodynamic and safety studies were performed with subjects that tended to have quite different demographics from the intended patient population. The applicable regulatory guideline for the conduct of pharmacokinetic studies in target species (EMEA/CVMP/133/99) suggests that studies should be conducted with 'animals of the target population'. The population pharmacokinetic modeling described in this study allowed the necessary characterization of mavacoxib pharmacokinetics in osteoarthritic patients. In studies with young adult Beagle dogs, mavacoxib exhibited broad BSV in pharmacokinetics (Cox *et al.*, 2010). The observation of this variability provided an additional rationale for use of population pharmacokinetic modeling as a tool to answer potential questions from the field trials about efficacy and/or safety.

In Study 1, the timing of drug administration with regard to meals was not controlled and data were not collected to indicate the frequency of drug administration with food. In Study 2, however, all doses were administered with food. Food was shown to increase mavacoxib bioavailability in laboratory Beagle dogs,

and the bioavailability of mavacoxib in Study 2 was therefore expected to be at least as good as in Study 1, so the model-predicted lower bioavailability (and net increase in Cl/F) in Study 2 was unexpected. In the laboratory Beagle dog study, the point estimate for the effect of a standardized meal of bioavailability was relatively imprecise, with a 90% confidence interval for the increase in bioavailability by the meal of 38–160% (Cox *et al.*, 2010). The meals presented to the patients in the field trials may have differed from the standardized meal used in the laboratory study and it is conceivable that the effect of food on bioavailability in field trials could have been relatively modest. Different lots of tablets were used in the two studies, but various laboratory tests of the tablets provided no reason to suspect lower bioavailability in Study 2. It is important to note that the difference in mavacoxib pharmacokinetics between the studies was coded for simplicity as a difference in bioavailability as Cl/F and V_d/F were similarly affected, but other explanations, e.g. nonlinear pharmacokinetics may also have contributed to the difference in Cl/F between the studies. Although dose proportionality was concluded in a laboratory Beagle dog study with mavacoxib at doses of 2, 4 and 12 mg/kg BW, the study was not powered to detect modest nonproportionality and the value of Cl/F at the 2 mg/kg dose was numerically larger than the Cl/F estimates from the higher doses by 18–21% (Cox *et al.*, 2010).

A mixed-effect pharmacokinetic model was developed for mavacoxib pharmacokinetics in laboratory Beagle dogs, and simulations were performed with the model before the field trials were conducted to evaluate various scenarios for the administration of mavacoxib with/without meals on the population pharmacokinetic model parameters when information about the meals was not included in the model dataset (Supporting Information). These simulations indicated that not accounting for the effect of the meals on F could, in some instances, bias Cl/F and V_d/F and also inflate both the residual variability and BSV. If individual patients consistently received their doses with food but these subjects were not identified in the model, residual variability might not be affected, but BSV could be inflated and both Cl/F and V_d/F would be decreased relative to drug administration in the fasted state. This decrease in Cl/F and V_d/F was a function of the fraction of patients receiving their doses with food and the ratio of fasting to fed bioavailability.[3] In the preliminary modeling with the data from the two studies, Cl/F and V_d/F were both increased in Study 2 but with little change in residual variability or the BSV in Cl/F. Overall, there is little evidence to indicate that the difference in mavacoxib pharmacokinetics between the studies is an artifact arising from a failure to account for the influence of food on bioavailability. Regardless of the explanation for the difference in pharmacokinetics between the studies, the studies appeared to have similar distributions of empirical Bayesian estimates for half-life.

In the final population pharmacokinetic model for mavacoxib, BW, age and breed were associated with mavacoxib Cl/F, and BW was associated with mavacoxib V_d/F. BW had the most profound effect of covariates on mavacoxib Cl/F and reduced the BSV in Cl/F from 65.4% to 49.3%. After accounting for the significant explanatory variables in the final population pharmacokinetic model, the BSV in Cl/F remained relatively large, at 46.9%, and the covariates explained relatively little of the BSV in Cl/F. Because of the relatively large BSV in pharmacokinetics and the relatively small reduction in variability provided by accounting for breed and age effects, dose adjustments based on breed and age are not likely to be useful in reducing the BSV in pharmacokinetics for typical geriatric large-breed osteoarthritic patients. The relatively large BSV in Cl/F was much larger than that of V_d/F, and the BSV in $t_{1/2}$ is also primarily related to the variability in Cl/F; patients with prolonged $t_{1/2}$ tended to have unusually low Cl/F. An allometric power function was used in the model to account for influences of the continuous covariate variables on Cl/F and V_d/F, and the coefficients for BW on Cl/F and V_d/F were approximately 0.75 and 1.0, respectively. These values are in excellent agreement with values reported for other studies with large ranges of BW (Anderson & Holford, 2008).

The pharmacokinetics of mavacoxib differs considerably between young adult laboratory Beagle dogs (or Beagle-sized Mongrel dogs) and the typical geriatric large-breed osteoarthritic patients. The typical Beagle $t_{1/2}$ is estimated to range from about 15 days (Supporting Information) to 17 days (Cox *et al.*, 2010) but the typical osteoarthritic patient $t_{1/2}$ (i.e. the $t_{1/2}$ in a 35 kg, 10-year-old patient that is not a German shepherd or Labrador retriever) is 44 days. The population pharmacokinetic model provides an indication that this difference in pharmacokinetics between the subject groups is primarily related to differences in the groups with regard to age and BW. The population pharmacokinetic model predicts a typical $t_{1/2}$ of 21 days in 1-year-old, 10 kg laboratory dogs, an estimate that is in reasonably good agreement with the observed data. The model also predicts that young adult laboratory Beagle dogs (1–2 years old) will have a $t_{1/2}$ that is typically 29–39% shorter than that of an identically sized 10-year-old osteoarthritic patient. Although geriatric pharmacokinetic data are quite limited for veterinary drugs, advanced age has been associated with decreased hepatic clearance of many drugs in humans and has been attributed to factors such as decreased activity of certain cytochrome P450 enzymes, decreased liver size, decreased diffusion of drugs into the liver, an increased extent of hepatocyte hypoxia, decreased hepatic blood flow for high clearance drugs, and the influence of age-associated conditions such as disease, frailty and stress. Drug absorptive capacity has been found to be generally unchanged in geriatric human subjects, but bioavailability may be increased for some drugs due to reduced presystemic hepatic extraction (Cusack, 2004; Benedetti *et al.*, 2007; Wauthier *et al.*, 2007). P-glycoprotein activity may be affected by age (Mangoni, 2007). Inflammation may also affect the activity of cytochrome P450

[3] Cl/F for the trial (Cl/F_{trial}) is given by the following equation: $Cl/F_{trial} = Cl/F_{fed} \times p_{fed} + Cl/F_{fasting} \times (1 - p_{fed})$. In the equation, Cl/F_{fed} and $Cl/F_{fasting}$ are oral clearances in fed and fasted states, respectively, and p_{fed} is the fraction of patients receiving all of their doses with food. Rearrangement yields the following equation: $Cl/F_{trial}/Cl/F_{fasting} = p_{fed} \times F_{fast}/F_{fed} + (1 - p_{fed})$. If F_{fast}/F_{fed} is 0.5 and $p_{fed} = 0.5$, the apparent Cl/F_{trial} would be 75% of $Cl/F_{fasting}$.

enzymes and transporters (Le Vee, 2009; Renton, 2005), and it is conceivable that the cytochrome P450 isoforms and transporters involved in the clearance of mavacoxib could have lower activity in geriatric osteoarthritic patients than in young adult healthy laboratory dogs. To the authors' knowledge, this is the first large-scale study to assess the effect of advanced age and/or disease on the pharmacokinetics of a veterinary drug.

Approximately 5% of the osteoarthritic patients were found to have an unusually prolonged $t_{1/2}$, i.e. $t_{1/2} > 80$ days and at least twice the typical $t_{1/2}$. No covariate factor was associated with prolonged $t_{1/2}$, and the long $t_{1/2}$ patients spanned a diverse range of BWs, breeds and ages. These animals in Study 1 had not reached steady state after seven administrations (6.5 months) of mavacoxib. In order to minimize potential safety risks associated with a continuous increase of mavacoxib plasma concentrations, Trocoxil is only registered for seven continuous administrations. Mavacoxib also exhibited broad variability in pharmacokinetics in the studies with laboratory Beagle dogs, and 3 of 63 beagles (4.8%) had $t_{1/2}$ values that were >30 days or approximately twice the typical $t_{1/2}$ of 15–17 days. It is possible, therefore, that factors associated with prolonged $t_{1/2}$ in laboratory Beagle dogs, e.g. a polymorphism of a transporter involved in the biliary clearance of mavacoxib, could also be predictive of prolonged $t_{1/2}$ in geriatric large-breed osteoarthritic patients.

In the model building work, no influence was noted on *Cl/F* for sex, any classes of concomitant medications, other breeds of patients, or laboratory tests of renal or hepatic function. It is important to note that discriminatory power to detect altered *Cl/F* in breeds other than German shepherds or Labrador retrievers may be limited by the relatively small numbers of patients in these other breed categories. No influence was anticipated for renal function tests on *Cl/F* as mavacoxib is primarily cleared by biliary excretion of unchanged drug (Hummel *et al.*, 2010). Furthermore, patients with impaired renal function were not enrolled in the study and few patients had serum creatinine or blood urea nitrogen test results outside of the normal range. The indirect indicators of liver function that were monitored during the studies (i.e. serum aspartate aminotransferase, alanine aminotransferase, ALKP and total bilirubin levels) were not found to influence mavacoxib *Cl/F*. Most of the patients had normal liver function tests, however, and no inference should be made from this analysis about mavacoxib clearance in patients with abnormal liver function tests. There were no drug interactions during the study that resulted in obvious changes in mavacoxib *Cl/F*. Mavacoxib is unlikely to be susceptible to clinically important pharmacokinetic drug interactions, however, as prolonged treatment with an interactant would likely be necessary to elicit an important change in mavacoxib *Cl/F*, and interactants that exclusively alter metabolic activity are not likely to result in a substantial change in *Cl/F* as the drug is primarily excreted intact in bile. Plasma protein-binding interactions are also unlikely for this orally administered low extraction ratio drug (Benet & Hoener, 2002).

This difference in pharmacokinetics between laboratory Beagle dogs and patients was recognized during the conduct of Study 1 and resulted in a reassessment of the dose required for efficacy and the margin of safety for the drug. Previous dose determination studies indicated that plasma mavacoxib trough concentrations above approximately 0.4 μg/mL were associated with efficacy (Lees *et al.*, 2009), and simulations with the population pharmacokinetic data from Study 1 indicated that a lower dose should provide efficacy. Based on the population pharmacokinetic data, the second pivotal field study (Study 2) was performed with a 50% reduced dose of 2 mg/kg BW. Target animal safety studies conducted in young adult healthy laboratory Mongrel dogs demonstrated a repeated dose (7 administrations) of 15 mg/kg BW to be well tolerated, whereas a dose of 25 mg/kg BW, with trough mavacoxib plasma concentrations exceeding 5 μg/mL, caused serious gastrointestinal ulcerations (EMEA, 2008, Krautmann *et al.*, 2009). Based on the results from the population pharmacokinetic analyses, the results from the target animal safety program and the field safety and efficacy data at 2 mg/kg BW, mavacoxib was approved at this reduced dose (EMEA, 2008; Payne-Johnson *et al.*, 2009). The results presented here indicate that the conduct of population pharmacokinetics can be a powerful tool in identifying the optimal dosage regimen. Given the relatively large BSV in mavacoxib pharmacokinetics in osteoarthritic patients, it is also likely that this population pharmacokinetic analysis provided a more relevant characterization of patient pharmacokinetics than a classical study with intensive blood sampling, but with a relatively small number of patients.

SUPPORTING INFORMATION

Additional Supporting information may be found in a online version of this article.

Fig. S1. Basic goodness of fit plots for the final model.

Table S1. Final model parameter values

Table S2. Summary of results for NONMEM fits of simulated data from 500 field safety and efficacy trials with mavacoxib 4 mg/kg BW, 200 patients per trial, and trough pharmacokinetic samples from each patient after doses 1, 2 and 4

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