JOURNAL OF
Veterinary Pharmacology and Therapeutics

J. vet. Pharmacol. Therap. doi: 10.1111/jvp.12087

The pharmacokinetics of oclacitinib maleate, a Janus kinase inhibitor, in the dog

W. T. COLLARD*
B. D. HUMMEL*
A. F. FIELDER*
V. L. KING*
J. F. BOUCHER*
M. A. MULLINS*
P. B. MALPAS* &
M. R. STEGEMANN†

*Veterinary Medicine Research and Development, Zoetis, Kalamazoo, MI, USA; †Veterinary Medicine Research and Development, Zoetis, Zaventem, Belgium

Collard, W. T., Hummel, B. D., Fielder, A. F., King, V. L., Boucher, J. F., Mullins, M. A., Malpas, P. B., Stegemann, M. R. The pharmacokinetics of oclacitinib maleate, a Janus kinase inhibitor, in the dog. *J. vet. Pharmacol. Therap.* doi: 10.1111/jvp.12087.

The pharmacokinetics of oclacitinib maleate was evaluated in four separate studies. The absolute bioavailability study used a crossover design with 10 dogs. The effect of food on bioavailability was investigated in a crossover study with 18 dogs. The breed effect on pharmacokinetics was assessed in a crossover study in beagles and mongrels dogs. Dose proportionality and multiple dose pharmacokinetics were evaluated in a parallel design study with eight dogs per group. In all four studies, serial blood samples for plasma were collected. Oclacitinib maleate was rapidly and well absorbed following oral administration, with a time to peak plasma concentration of <1 h and an absolute bioavailability of 89%. The prandial state of dogs did not significantly affect the rate or extent of absorption of oclacitinib maleate when dosed orally, as demonstrated by the lack of significant differences in pharmacokinetic parameters between the oral fasted and oral fed treatment groups. The pharmacokinetics of oclacitinib in laboratory populations of beagles and mixed breed dogs also appeared similar. Following oral administration, the exposure of oclacitinib maleate increased dose proportionally from 0.6 to 3.0 mg/kg. Additionally, across the pharmacokinetic studies, there were no apparent differences in oclacitinib pharmacokinetics attributable to sex.

(Paper received 29 March 2013; accepted for publication 4 October 2013)

Wendy Collard, Veterinary Medicine Research and Development, Zoetis, 333 Portage Street, Kalamazoo, MI 49007, USA. E-mail: Wendy.Collard@Zoetis.com

INTRODUCTION

APOQUEL® (oclacitinib maleate) was the first selective Janus kinase (JAK) inhibitor to be developed for dogs. It was recently approved for the control of pruritus associated with allergic dermatitis and control of atopic dermatitis in dogs at least 12 months of age. In recent years, there has been a significant evolution in understanding regarding the pathophysiology of allergic and atopic diseases. The molecular mechanisms involved in stimulating the itch response have been elaborated (Marsella *et al.*, 2012), and it is now understood that cytokines play a key role in initiating the neuronal itch stimulation which triggers pruritic behavior (scratching, rubbing, chewing, etc.) in dogs. This leads to the establishment of a vicious cycle of itch that exacerbates skin lesions and amplifies defects in the skin barrier function in clinically affected dogs. This progress provided the opportunity to develop new treatments (Marsella *et al.*, 2012). Specifically, Janus kinases play a central role in cytokine signaling and are involved in signal transduction of many pro-inflammatory, pro-allergic, and pruritogenic cytokines including IL-2, IL-4, IL-6, IL-13, and IL-31 that are implicated in allergic conditions (Ong & Leung, 2006; Carmi-Levy *et al.*, 2011).

APOQUEL was selected for development, in part, because in cellular assays, it preferentially inhibits JAK1-dependent cytokines involved in allergy, inflammation, and pruritus (Gonzales *et al.*, 2013) over JAK2-dependent cytokines which are associated with changes in the hematopoietic system (Ghoreschi *et al.*, 2009; Quintas-Cardama *et al.*, 2011). Additionally, oclacitinib's inhibitory effect on IL-31, a cytokine which plays a key role in canine pruritus, was demonstrated recently in IL-31 and flea-associated pruritus models as well as in naturally diseased animals (Fleck *et al.*, 2012; Gonzales *et al.*, 2012).

An APOQUEL clinical field study in client-owned dogs suffering from pruritus associated with allergic dermatitis demonstrated that a twice a day dose of 0.4–0.6 mg oclacitinib/kg provided a rapid onset of efficacy with a reduction in pruritus within the first observation time at 24 h postadministration (Cosgrove *et al.*, 2013). Pruritus scores decreased from 'severe itching' to 'very mild itching' the first week of oclacitinib treatment. In another clinical field study, oclacitinib maleate was demonstrated to be safe and effective in the treatment of

© 2013 John Wiley & Sons Ltd

canine AD when administered at the targeted label dose of 0.4–0.6 mg/kg twice a day for 2 weeks and then reduced to 0.4–0.6 mg/kg once a day for maintenance therapy (Cosgrove *et al.*, accepted).

This manuscript describes the pharmacokinetics of oclacitinib in dogs as observed in four laboratory studies. Study 1 describes the absolute oral bioavailability of oclacitinib administered in the commercial tablet formulation. Study 2 determined whether prandial state affected the pharmacokinetics of orally administered oclacitinib. Study 3 compared the effect of dog breed (beagle vs. mongrel) on the intravenous and oral pharmacokinetics of oclacitinib. Finally, Study 4 evaluated oral pharmacokinetics of oclacitinib following multiple days of dosing and determined oral dose proportionality from 0.6 to 3.0 mg/kg.

MATERIALS AND METHODS

Pharmacokinetic studies

These studies were conducted in accordance with regulations outlined in the USDA Animal Welfare Act (9 CFR Parts 1, 2, and 3) and the conditions specified in *Guide for the Care and Use of Laboratory Animals* (National Academy Press, Washington DC, 1996).

Oclacitinib was studied as the maleate salt. All doses are expressed in terms of mg/kg of free base. Studies 1 and 4 used the commercial tablet formulation. Studies 2 and 3 used bulk drug and microcellulose in gelatin capsules. In all four studies, serial blood samples for determination of pharmacokinetics were collected following oclacitinib administration. Blood samples were collected via jugular venipuncture into K_2 EDTA tubes and placed on ice until centrifuged. Harvested plasma was stored at approximately −20 °C until analysis, and all samples were analyzed within the long-term sample stability limit established according the FDA guidance 145). The single dose studies (Studies 1–3) collected blood samples at 0, 0.03, 0.5, 1, 2, 4, 8, 12, 24, 32, and 48 h postdose. Sampling times were more limited for the multiple dose study (Study 4), with blood samples collected at 0, 1, 4, 8, and 12 h after the first of the two daily doses on Days 0, 21, 53, and 168. For all studies, the blood samples were considered to be on time if the actual sampling times were within ±10% of the prescribed time and ±1 h for the 24, 32, and 48 h samples.

Study 1: bioavailability

Ten beagle dogs (5 male, 5 female) (Marshall BioResources, North Rose, NY, USA) were used in a two-period, two-treatment GLP (Good Laboratory Study) crossover study design with two sequences. The dogs were approximately 8 months of age, 6.25–10.9 kg at the time of enrollment and were randomly assigned to treatment sequence. The treatments were a single intravenous (i.v) 0.282 mg/kg bolus dose and a single 0.4–0.6 mg/kg oral dose. The periods were separated by a one-week washout period, and dogs received each treatment only once. For the i.v treatment, the oclacitinib solution (20% 2-hydroxypropyl-β-cyclodextrin in sterile water and 3.8 mg/mL oclacitinib) was administered via the cephalic vein as an i.v bolus. For the oral treatment, dogs received a single 3.6 mg or 5.4 mg oclacitinib tablet depending on body weight to result in administration of a 0.4–0.6 mg/kg dose. For both treatments, animals were fasted overnight prior to dosing and were fed 4 h postdose. The oral pharmacokinetic parameters for each dog, C_{max}, $AUC_{0\text{-}t(last)}$, and $AUC_{0\text{-}\infty}$, were normalized to 0.4 mg/kg.

Study 2: food effect

Eighteen beagle dogs (9 male, 9 female) (Marshall BioResources) were used in a three-period, three-treatment crossover study design that had six sequences such that each dog received each treatment only once. The dogs were between 1 and 5 years of age, 7.5–13.3 kg at the time of enrollment and were blocked on body weight into blocks of six dogs and within block randomly assigned to treatment sequence. The treatments included a single i.v bolus dose of 0.5 mg/kg, a single oral dose of 0.5 mg/kg administered in the fasted state and a single oral dose of 0.5 mg/kg administered in the fed state. Food was withheld for the i.v and fasted treatments overnight and until approximately 4 h postdose. Food (50 g of Hills Prescription Diet p/d (canned dog food) plus approximately 250 g of Purina Lab Canine Diet 5007) was provided for the fed treatment 30 min prior to dose administration, and food consumption was measured 1 h postdose. The periods were separated by a one-week washout. For the i.v treatment, the oclacitinib solution (20% 2-hydroxypropyl-β-cyclodextrin in sterile water and 5.0 mg/mL oclacitinib) was administered via the cephalic vein as an i.v bolus using a dose volume of 0.1 mL/kg. For the oral treatment, dogs received a single gelatin capsule (hand filled with oclacitinib maleate and microcrystalline cellulose) to result in a 0.5 mg/kg dose.

Study 3: breed effect

Thirty dogs (young adult to adult) were used in a crossover study with two periods and two treatments for both beagles (Covance, Madison, WI, USA) and mongrels (Covance). Each dog received each treatment only once during the study. Fifteen beagle dogs (8 male and 7 female) weighing from 10.0 to 12.5 kg and fifteen mongrel dogs (8 male and 7 female) weighing from 20.5 to 37.5 kg were used in the study. Due to facility constraints, beagle and mongrel dogs were housed in separate rooms and were randomized separately within sex to treatment sequence. The treatments included a single i.v bolus dose of 0.4 mg/kg and a single oral dose of 0.4 mg/kg. Food was withheld overnight and until approximately 4 h postdose. The periods were separated by a one-week washout. For the i.v treatment, the oclacitinib solution (4.0 mg/mL oclacitinib solution in 20% Cavitron vehicle (hydroxypropyl-B-cyclodextrin), pH7.0) (Cerestar, Hammond, IN, USA) was administered via the cephalic vein as an i.v bolus using a dose volume of 0.1 mL/kg. For the oral treatment, dogs received a single

© 2013 John Wiley & Sons Ltd

gelatin capsule (hand filled with oclacitinib maleate and microcrystalline cellulose) to result in a 0.4 mg/kg dose.

Study 4: multiple dose and dose proportionality

The multiple dose and dose proportionality was evaluated in a GLP margin of safety study. The study aspects relating to the pharmacokinetic analysis are described here and the safety aspects will be reported elsewhere. Thirty-two beagle dogs (16 female, 16 male) (Marshall BioResources) were allocated to four treatment groups. Dogs in Treatment 1 received empty placebo capsule (0 mg/kg) orally. Dogs in Treatments 2, 3, and 4 received a combination of whole and half 3.6, 5.4, and 16 mg oclacitinib tablets orally to result in minimum target doses of 0.6, 1.8, and 3.0 mg/kg, respectively. All dogs received twice daily doses administered approximately 12 h apart of 0, 0.6, 1.8, and 3.0 mg/kg for weeks 1 through 6 and a single daily dose of 0, 0.6, 1.8, and 3.0 mg/kg for weeks 7 through 26. On pharmacokinetic sample collection days, dogs were fasted the previous night and fed 4 h postdose administration. To calculate AUC_{0-24} for study days 53 and 168, steady-state was assumed, and the 24-h concentration was assumed to be equal to the 0 h concentration and used for the 0 h concentration. As the actual individual doses of oclacitinib varied from the group target doses, the pharmacokinetic variables $AUC_{0-\tau}$ (AUC of the dosing interval), C_{max}, and C_{τ} (trough concentration) were all normalized to the group target dose.

Analytical methodology

Plasma samples from Studies 1, 3, and 4 were analyzed for concentrations of oclacitinib by a validated protein precipitation extraction method. A 50 μL volume of each sample, standard, or QC was transferred to a Costar tube in a 96-well plate format. 200 μL of internal standard, M + 4 stable label (Pfizer Inc., New York, NY, USA) (98.9% purity), (IS, 10 ng/mL) solution in acetonitrile was added to all samples. The plate was sealed, mixed by vortexing, and then centrifuged at approximately 100 ***g*** for 10 min for precipitation of proteins. A 20 μL volume of each sample was transferred to a clean 96-well plate, diluted with 180 μL of 0.1% ammonium hydroxide, sealed, and mixed gently. Then, the plate was placed in the autosampler for analysis by LC-MS/MS.

A 10 μL volume of each prepared sample was injected onto a 5 μm Zorbax Extend C18 50 × 2 mm HPLC column and eluted at 0.300 mL/min using a gradient. The HPLC mobile phases were A: 0.1% ammonium hydroxide in water (pH = 10.7) and B: 0.1% ammonium hydroxide in acetonitrile. The gradient went from 90% A/10% B to 70% A/30% B in 0.5 min and held at 70% A/30% B for 2 min. Detection was performed using a Sciex API 4000 mass spectrometer (MDS Sciex, Foster City, CA, USA) using positive electrospray ionization (ESI+) with multiple reaction monitoring of the transitions 338 → 149 amu for oclacitinib and 342 → 149 amu for M + 4 stable label. The retention times were approximately 3 min for oclacitinib and for the stable label, with a total run time of 5 min. Peak areas were used for quantitation with quadratic regression (1/×2 weighting). Samples above the upper limit of quantitation were re-assayed after dilution with blank dog plasma. The concentration range of the calibration curve was 0.5–1000 ng/mL.

Each analytical run contained duplicate standard curves ranging from 0.5 to 1000 ng/mL oclacitinib and quality control samples (n = 5 sets, minimum) at 1.0, 30, and 600 ng/mL. Integration of chromatograms was performed in Analyst Software (v1.4.2; Applied Biosystems, Foster City, CA, USA), and raw data (peak areas) were imported into Watson LIMS (v7.2.0.03; Thermo Fisher Scientific, Philadelphia, PA, USA) for standard regression. Intrarun bias of standards ranged from −7.5 to 6.0% including samples at the (lower limit of quantitation) LLOQ (0.5 ng/mL), and intrarun bias of quality control samples ranged from −2.0 to 9.0%. The inter-run coefficient of variation ranged from 0.7 to 16.7% for standards and 0.4–13.2% for quality control samples.

Plasma samples from Study 2 were analyzed using a nearly identical method as above, with the exception that a close structural analog was used as the internal standard (transition 378 → 149 amu, Pfizer, Inc., >95% purity), and the standard curve range was 0.1–1000 ng/mL.

Data analysis

Plasma concentration. To determine means and confidence intervals for the oclacitinib plasma concentration data, the log-transformed concentrations were analyzed using a repeated-measure analysis of variance. The factors included in the analysis varied based on the study design. Plasma concentrations that were below the lower limit of quantitation (LLOQ) were included as one half the LLOQ if greater than half of the values were above the LLOQ. If greater than half of the plasma concentration values for a given time point were below the LLOQ, then the mean and confidence intervals were not reported.

Non-compartmental pharmacokinetic calculations and statistical analysis

Pharmacokinetic calculations were performed on the plasma concentration data from the studies using noncompartmental analysis with Watson version 7.2.0.03. The actual collection times did not vary beyond the prescribed times, thus calculations used nominal times. Estimates of the area under the plasma concentration vs. time profile (AUC) were determined by the linear trapezoidal method with extrapolated area determined using the slope of the log-linear phase. Estimates for the observed maximum plasma concentration (C_{max}), time to maximum concentration (t_{max}), $AUC_{0-t(last)}$, $AUC_{0-\infty,}$ and terminal half-life ($t½$) were determined for all animals. For animals receiving i.v administration, concentration at time 0 (C_0) was determined by back extrapolating from a linear regression of the first two time points. The total body clearance (CL) ($CL = Dose/AUC_{0-\infty}$)and apparent volume of distribution at steady-state (Vd_{ss}) (Vdss = CL*MRT) were also calculated in

© 2013 John Wiley & Sons Ltd

Watson. Plasma concentrations that were below the LLOQ were excluded from the pharmacokinetic analysis.

In Study 1, the log-transformed $AUC_{0\text{-}\infty}$ values were analyzed using the model described below to estimate the bioavailability. The 0.282 mg/kg i.v $AUC_{0\text{-}\infty}$ values were dose adjusted to 0.4 mg/kg assuming dose proportionality. The difference between the mean $AUC_{0\text{-}\infty}$ values from the oral and i.v treatments on the log-transformed scale was calculated. Back-transforming the difference resulted in the estimate of absolute bioavailability.

The means and confidence intervals (CI) associated with the pharmacokinetic parameters were estimated using a mixed effects linear model. The parameters C_{max}, CL, Vd_{ss}, $AUC_{0\text{-}t(last)}$, and $AUC_{0\text{-}\infty}$ were log-transformed prior to analysis, and $t_{½}$ was inverse-transformed prior to analysis. For Study 1 and Study 3, the model included the fixed effects of sequence, period, and treatment, and the random effects of animal and within animal error. For Study 2, the model included the fixed effects of period, treatment, and carryover; and random effects of block, animal (block), and within animal error. For Study 4, the model included the fixed effects of sex, treatment, and sex by treatment interaction and random effects of block (sex) and between animal errors. Back-transformed means and 95% CI were constructed and reported.

To test for dose proportionality in Study 4, a previously described method was utilized (Gough *et al.*, 1995; Klamerus *et al.*, 1992; Smith *et al.*, 2000; Hummel *et al.*, 2009). Briefly, C_{max} and $AUC_{0\text{-}\infty}$ were fit to the model: $Log(Y) = \alpha + \beta \bullet Log(dose)$. This relationship can also be expressed as, $Y = \alpha \bullet Dose^{\beta}$, where $Y = C_{max}$ or $AUC_{0\text{-}\infty}$, α is dependent on other terms in the model and β is a proportionality constant which equals 1 if dose proportionality holds. The model included the fixed effect terms for sex, log (dose), lack of fit (dropped from the model if not significant) and random effect terms for block (sex) and error. The β parameter was estimated, and a 90% confidence interval was determined. Dose proportionality was concluded if the 90% confidence interval on the estimate of β included 1 and if the test for lack of fit was not significant ($P > 0.05$).

RESULTS

Administration of oclacitinib was well tolerated in all treatment groups in all studies. No animals were withdrawn from the studies, and no serious adverse reactions were observed.

Study 1: bioavailability

Least-squares mean plasma concentration–time profiles of oclacitinib following i.v administration of 0.282 mg/kg and oral administration of 0.4–0.6 mg/kg dose are shown in Fig. 1. The estimates of the noncompartmental pharmacokinetic parameters following i.v administration and a single oral administration of oclacitinib maleate are summarized in Table 1. The blood sampling was adequate to characterize the pharmacokinetic profile, as the extrapolated AUC was within 1% of the $AUC_{0\text{-}t(last)}$. Oclacitinib maleate was well absorbed from the proposed commercial tablets with an absolute oral bioavailability of 89% (95% CI 85–94%). There were no apparent differences between males and females in plasma concentrations or pharmacokinetic parameters.

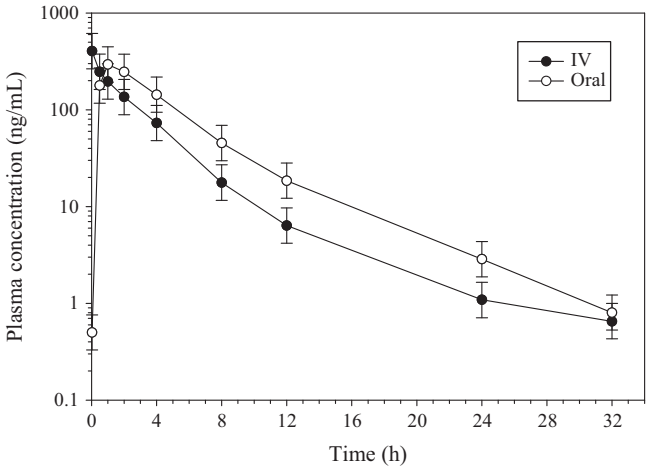

Fig. 1. Least-squares mean and 95% confidence interval plasma concentration–time profiles of oclacitinib in beagle dogs following either a single intravenous administration of 0.282 mg/kg or oral administration of 0.4–0.6 mg/kg oclacitinib. Values below the limit of quantification (LOQ) were replaced with ½ LOQ (0.5 ng/mL); note that at 48 h all of the dogs except for one dog in the i.v treatment group and one in the oral treatment were below the LOQ.

Study 2: food effect

Following oral administration in the fed state, all subjects with the exception of one dog consumed >25% of their daily ration of food within 1 h of dose administration. This subject consumed approximately 2% of food in this time period. All subjects were included in the analyses. Least-squares mean plasma concentration–time profiles of oclacitinib following i.v administration and oral administration in the fasted and fed state at a dose of 0.5 mg/kg are shown in Fig. 2. The estimates of the noncompartmental pharmacokinetic parameters following i.v administration and a single oral administration of oclacitinib are summarized in Table 1. The blood sampling was adequate to characterize the pharmacokinetic profile, as the extrapolated AUC was within 1% of the $AUC_{0\text{-}t(last)}$. Oclacitinib maleate was well absorbed with an absolute oral bioavailability of $85 \pm 24.9\%$ when dosed to fasted dogs and $79 \pm 27.9\%$ when dosed to fed dogs. There were no significant differences at the 10% level of significance ($P < 0.10$) in t_{max}, C_{max}, $AUC_{0\text{-}t(last)}$, or $AUC_{0\text{-}\infty}$ between the oral fasted and oral fed treatment groups. There were no apparent differences between males and females in plasma concentrations or pharmacokinetic parameters.

Study 3: breed effect

The least-squares mean for the pharmacokinetic variables are presented in Table 1, and the least-squares mean plasma

© 2013 John Wiley & Sons Ltd

Table 1. Summary of LS mean pharmacokinetic parameters (95% confidence intervals) following either single i.v or oral administration of oclacitinib to dogs

	Study 1		Study 2			Study 3			
PK Parameter	i.v	p.o	i.v (fasted)	p.o (fasted)	p.o (fed)	i.v (beagle)	i.v (mongrel)	p.o (beagle)	p.o (mongrel)
Dose (mg/kg)	0.282	0.4–0.6	0.5	0.5	0.5	0.4	0.4	0.4	0.4
$AUC_{0\text{-}t(last)}$ (ng·h/mL)	948 (708, 1271)	1197* (902, 1589)	3521 (2409, 5146)	2264 (1517, 3376)	2159 (1510, 3087)	1733 (1442, 2082)	1866 (1588, 2191)	1576 (1311, 1894)	1545 (1315, 1815)
$AUC_{0\text{-}\infty}$ (ng·h/mL)	953 (713, 1275)	1206* (909, 1599)	3535 (2650, 4719)	2262 (1695, 3019)	2164 (1622, 3088)	1739 (1447, 2090)	1871 (1593, 2196)	1591 (1324, 1912)	1556 (1324, 1827)
C_{max} (ng/mL)	NC	259* (189, 355)	6.0 (5.5, 6.7)	372 (302, 459)	378 (297, 481)	NC	NC	259 (181, 370)	267 (228, 312)
t_{max} (h)	NC	0.9 (0.46, 1.34)	NC	1.2 (0.8, 1.6)	0.9 (0.6, 1.3)	NC	NC	0.99 (0.15, 1.83)	1.2 (0.57, 1.84)
t½ (h)	3.45 (2.21, 4.68)	4.13 (3.08, 5.19)	NC	5.1 (4.7, 5.6)	5.9 (5.1, 6.9)	4.02 (3.42, 4.87)	4.13 (3.69, 4.68)	4.0 (3.4, 4.9)	4.3 (3.8, 5.0)
CL†(mL/min/kg)	5.27 (3.95, 6.6)	NC	2.45 (2.01, 2.90)	NC	NC	4.05 (2.64, 5.46)	3.66 (3.19, 4.12)	NC	NC
Vdss†(L/kg)	0.942 (0.870, 1.01)	NC	0.66 (0.59, 0.73),	NC	NC	1.07 (0.928, 1.21)	0.939 (0.833, 1.04)	NC	NC

*Dose normalized to 0.4 mg/kg. †Geometric mean. NC, not calculated.

concentrations are illustrated in Fig. 3. The plasma sampling scheme provided sufficient data to characterize $AUC_{0\text{-}\infty}$, based on the percent extrapolation. There were no apparent differences between males and females in plasma concentrations or pharmacokinetic parameters. The plasma concentration–time profiles and the pharmacokinetic parameters following i.v and p.o administration to beagle and mongrel dogs were very similar. Although a statistical test for equivalence was not performed due to the inability to randomize beagles and mongrels between rooms, the similarity of the means and the overlap of the confidence intervals following both i.v and oral administration demonstrated that breed does not impact the pharmacokinetic profile of oclacitinib.

Fig. 2. Least-squares mean plasma concentration–time profiles of oclacitinib in beagle dogs following either a single intravenous administration of 0.5 mg/kg or oral administration of 0.5 mg/kg oclacitinib to fed or fasted dogs.

Fig. 3. Least-squares mean plasma concentration–time profile of oclacitinib in beagle and mongrel dogs following either a single intravenous administration of 0.4 mg/kg or oral administration of 0.4 mg/kg oclacitinib.

© 2013 John Wiley & Sons Ltd

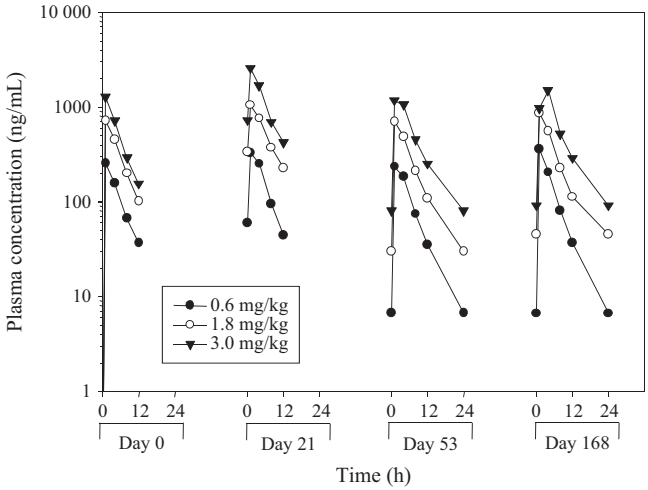

Fig. 4. Least-squares mean plasma concentration–time profiles of oclacitinib in beagle dogs following oral twice daily administration (Day 0, 21) and once daily administration (Day 53, 168) of 0.6 mg/kg, 1.8 mg/kg, and 3.0 mg/kg.

Study 4: multiple dose and dose proportionality

Least-squares mean plasma concentration–time profiles of oclacitinib following oral administration of 0, 0.6, 1.8, and 3.0 mg/kg for 168 days are shown in Fig. 4. The actual doses for the 0.6 mg/kg nominal dose were 0.6–0.7 mg/kg, 1.8–1.9 for the 1.8 mg/kg nominal dose group, and 3.0–3.1 mg/kg for the 3.0 mg/kg nominal dose group. The estimates of the noncompartmental pharmacokinetic parameters on days 0, 21, 53, and 168 are summarized in Table 2. On Day 0, $AUC_{0–12}$ and C_{max} increased in a dose-related manner following oral administration of tablets dosed at target doses of 0.6 mg/kg, 1.8 mg/kg, and 3.0 mg/kg. The increase in $AUC_{0–12}$ and C_{max} was dose proportional from 0.6 to 3.0 mg/kg.

Across all days and doses, there did not appear to be any systematic male/female differences in pharmacokinetic parameters. Plasma exposure increased with the number of doses following twice a day administration with a significant difference at the 0.10 concentration in $AUC_{0–12}$ for study Day 0 in comparison with Day 21. There was a numerical decrease in the plasma exposure over the 24-h period following the change in dosage regimen to once a day on Day 43. The Day 0 and Day 53 least-squares mean values for C_{max} were not significantly different for 0.6, 1.8, and 3.0 mg/kg. Day 53 and Day 168 the least-squares mean values for C_{max} and $AUC_{0–24}$ were not significantly different for 1.8 and 3.0 mg/kg. While the Day 53 and Day 168 least-squares mean values for C_{max} and $AUC_{0–24}$ following 0.6 mg/kg once a day were significantly different, the C_τ values were not.

DISCUSSION

In all four studies, following oral administration oclacitinib was rapidly absorbed in dogs with mean maximum plasma concentrations occurring at approximately 1 h postdose. This absorption is consistent with the observed rapid onset of pruritus reduction in both laboratory and clinical field studies with APOQUEL (Fleck *et al.*, 2012; Cosgrove *et al.*,2013). Oclacitinib was shown to be a low clearance compound as defined by Toutain (Toutain & Bousquet-Melou, 2004) with a moderate volume of distribution. The absolute oral bioavailability was high with a mean range of 79% to 89%. Furthermore, it can be concluded that the absorption is nearly complete based on the calculated fraction absorbed of >0.9 (based on the data presented in Table 1; bioavailability of 85%, mean clearance of 4 mL/min/kg, and liver blood flow of 40 mL/min/kg). The observed increase in mean $AUC_{0–12}$ from Day 0 to Day 21 of 40% was slightly greater than the expected increase in approximately 15% based on plasma elimination half-life ($t½$) of 4 h. Assuming a $t½$ of 4 h, it would be predicted that steady-state would be achieved by the second dose following the dosage

Table 2. Summary of LS mean pharmacokinetic parameters following oral administration of 0, 0.6, 1.8, and 3.0 mg/kg oclacitinib twice a day (BID) the first 6 weeks and then once a day (SID) for an additional 20 weeks

Pharmacokinetic Variable	Treatment	Day	LS mean	90% CI
C_{max} (ng/mL)	0.6 mg/kg BID	0	238	195–290
		21	328	269–400
	0.6 mg/kg SID	53	255	209–310
		168	333	273–406
	1.8 mg/kg BID	0	708	580–863
		21	1030	844–1260
	1.8 mg/kg SID	53	711	583–867
		168	846	694–1030
	3.0 mg/kg BID	0	1260	1040–1540
		21	2570	2110–3130
	3.0 mg/kg SID	53	1380	1130–1680
		168	1610	1320–1960
t_{max} (h)	0.6 mg/kg BID	0	1	1–2
		21	2	1–3
	0.6 mg/kg SID	53	2	1–2
		168	1	0–2
	1.8 mg/kg BID	0	1	1–1
		21	1	1–1
	1.8 mg/kg SID	53	1	1–2
		168	1	1–1
	3.0 mg/kg BID	0	1	0–2
		21	1	0–2
	3.0 mg/kg SID	53	2	1–3
		168	3	2–4
$AUC_{0-\tau}$* (ng·h/mL)	0.6 mg/kg BID	0	1341	1060–1690
		21	1920	1520–2430
	0.6 mg/kg SID	53	1720	947–3130
		168	2020	1110–3680
	1.8 mg/kg BID	0	4050	3170–5160
		21	6800	5330–8680
	1.8 mg/kg SID	53	5100	2900–8960
		168	5790	3290–10 200
	3.0 mg/kg BID	0	6720	5080–8910
		21	15 000	11 300–19 900
	3.0 mg/kg SID	53	10 900	4840–24 500
		168	12 800	5680–28 700

*BID τ = 12 and SID τ = 24.

© 2013 John Wiley & Sons Ltd

regimen change from twice daily to once daily. The similarity of the observed pharmacokinetic parameters on Day 53 (change from twice a day to once a day was on day 43) and Day 168 at 0.6 mg/kg once a day supports this conclusion.

The observed pharmacokinetic parameters of rapid oral absorption and high bioavailability were consistent with the physicochemical properties of oclacitinib. The *in vitro* permeability of oclacitinib was experimentally determined in a Caco-2 cell monolayer study. The permeability was high, 40.4×10^{-6} cm/sec, greater than the control for high permeability (Zoetis internal data not shown). Additionally, the solubility of oclacitinib is pH dependent with a significant drop in solubility above pH 4 down to practically insoluble by pH 5.5 (Zoetis internal data not shown). The dog gastric pH in fed and fasted dogs is typically <4 (Sagawa *et al.*, 2009; Mahar *et al.*, 2012). At a pH of <4, the solubility of oclacitinib maleate (10.43 mg/mL at pH3.8) the dose for a 10 kg dog (6 mg) would fully dissolve in 0.6 mL. Although the estimation of the liquid volume to use for a dog is complex due to the relative small number of studies in the literature, the large size differences among individual dogs, and the lack of administration of water with doses, the suggested volumes for a 10 kg dog of 9–20 mL are well above what is needed for oclacitinib to be fully soluble (Martinez & Papich, 2012). The solubility profile of oclacitinib is also supportive of the lack of a prandial effect. Although the pH in the fed state has been shown to spike to around pH 7, a majority of the time the pH is 2–4 (Sagawa *et al.*, 2009; Mahar *et al.*, 2012). Thus, under both fed and fasted conditions, oclacitinib was expected to be fully dissolved in both states, further supporting the observed experimental result that oclacitinib, given with or without food results in a similar oral pharmacokinetic profile.

The pharmacokinetic analysis in beagles/mongrels and males/females does not predict any population differences. The plasma concentration–time profiles and the pharmacokinetic parameters following i.v and oral administration to beagle and mongrel dogs were very similar. Although a statistical test for equivalence was not performed due to the inability to randomize beagles and mongrels between rooms, the similarity of the means and the overlap of the confidence intervals following both i.v and oral administration leads to the conclusion that breed does not impact the pharmacokinetic profile. Although a formal population model was not developed, all the pharmacokinetic data predict that no clinically different pharmacokinetic profiles in client-owned dogs would be different from those reported here.

The four pharmacokinetic studies demonstrated that at the approved label dose of 0.4–0.6 mg oclacitinib per kg APOQUEL exhibits rapid and nearly complete absorption, low clearance, no pharmacokinetic differences in male, female, fed, fasted, beagle and mongrel dogs, and dose proportionality.

REFERENCES

Carmi-Levy, I., Homey, B., & Soumelis, V. (2011) A modular view of cytokine networks in atopic dermatitis. *Clinical Reviews in Allergy and Immunology*, **41**, 245–253.

Cosgrove, S.B., Wren, J.A., Cleaver, D.M., Martin, D.D., Walsh, K.F., Harfst, J. A., Follis, S.L., King, V.L., Boucher, J.F. & Stegemann, M.R. (2013) Efficacy and safety of oclacitinib in the treatment of pruritus and associated skin lesions in dogs with canine allergic dermatitis. *Veterinary Dermatology*, **24**, 479–e114.

Cosgrove, S.B., Wren, J.A., Cleaver, D.M., Walsh, K.F., Follis, S.L, King, V.L., Tena, J.S. & Stegemann, M.R. (accepted) A blinded randomized placebo-controlled trial of the efficacy and safety of the Janus kinase inhibitor oclacitinib (Apoquel®;) in client-owned dogs with atopic dermatitis. *Veterinary Dermatology*, **24**, 587–e142.

Fleck, T., Humphrey, W., Coscarelli, E., Galvan, B., Aleo, M., Gonzales, A., Shelly, J., Mahabir, S. & McCall, R. (2012) Comparison of the janus kinase (JAK) inhibitor, oclacitinib, and prednisolone in canine models of pruritus. *Veterinary Dermatology*, **23**(Suppl 1), 38.

Ghoreschi, K., Laurence, A. & O'Shea, J.J. (2009) Janus kinases in immune cell signaling. *Immunological Reviews*, **228**, 273–287.

Gonzales, A.J., Humphrey, W., Messamore, J.E., Fleck, T.J., Fici, G.J., Shelly, J.A., Teel, J.F., Bammert, G.F., Dunham, S.A., Fuller, T.E. & McCall, R.B. (2012) IL-31: its role in canine pruritus and prevalence in naturally occurring canine atopic dermatitis. *Veterinary Dermatology*, **23**(Suppl. 1), 6.

Gonzales, A.J., Bowman, J., Fici, G., Zhang, M. & Mann, D.M. (2013) Oclacitinib (Apoquel, Zoetis) is a novel Janus kinase inhibitor that has activity against canine pro-allergic and pro-inflammatory cytokines A. J. Gonzales, ESVD Abstract # 40 Valencia Spain September 2013. *Veterinary Dermatology* **24**, 384.

Gough, K., Hutchison, M., Keene, O., Byrom, B., Ellis, S., Lacey, L. & McKellar, J. (1995) Assessment of dose proportionality: report from the statisticians in the pharmaceutical industry/pharmacokinetics UK joint working party. *Drug Information Journal*, **29**, 1039–1048.

Guidance for industry 145: Bioanalytical method validation. U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER) and Center for Veterinary Medicine (CVM), May 2001

Hummel, J., McKendrick, S., Brindley, C. & French, R. (2009) Exploratory assessment of dose proportionality: Review of current approaches and proposal for a practical criterion. *Pharmaceutical Statistics*, **8**, 38–49.

Klamerus, K.J., Maloney, K., Rudolph, R.L., Sisenwine, S.F., Jusko, W.J. & Chiang, S.T. (1992) Introduction of a composite parameter to the pharmacokinetics of Venalfaxine and its active O-desmethyl metabolite. *Journal of Clinical Pharmacology*, **32**, 716–724.

Mahar, K.M., Portelli, S., Coatney, R. & Chen, E.P. (2012) Gastric pH and gastric residence time in fasted and fed conscious beagle dogs using the Bravo pH system. *Journal of Pharmaceutical Sciences*, **101**, 2439–2448.

Marsella, R., Sousa, C.A., Gonzales, A.J. & Fadok, V.A. (2012) Current understanding of the pathophysiologic mechanisms of canine atopic dermatitis. *Journal of the American Veterinary Medical Association*, **241**, 194–207.

Martinez, M.N. & Papich, M.G. (2012) Drug solubility classification in the dog. *Journal of Veterinary Pharmacology and Therapeutics*, **35**(Suppl 1), 87–91.

Ong, P.Y. & Leung, D.Y. (2006) Immune dysregulation in atopic dermatitis. *Current Allergy and Asthma Reports*, **6**, 384–389.

Quintás-Cardama, A., Kantarjian, H., Cortes, J. & Verstovsek, S. (2011) Janus kinas inhibitors for the treatment of myeloproliferative neoplasias and beyond. *Nature Reviews Drug Discovery*, **10**, 127–140.

Sagawa, K., Li, F., Liese, R. & Sutton, S.C. (2009) Fed and fasted gastric pH and gastric residence time in conscious beagle dogs. *Journal of Pharmaceutical Sciences*, **98**, 2494–2500.

Smith, B.P., Vandenhende, F.R., DeSante, K.A., Farid, N.A., Welch, P.A., Callaghan, J.T. & Forgue, S.T. (2000) Confidence Interval Criteria for Assessment of Dose Proportionality. *Pharmaceutical Research*, **17**, 1278–1283.

Toutain, P.L. & Bousquet-Melou, A. (2004) Plasma clearance. *Journal of Veterinary Pharmacology and Therapeutics*, **27**, 415–425.

© 2013 John Wiley & Sons Ltd