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# Pharmacokinetics, pharmacodynamics, toxicology and therapeutics of mavacoxib in the dog: a review

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Mavacoxib is a novel nonsteroidal anti-inflammatory drug (NSAID), with a preferential action on the cyclooxygenase (COX)-2 isoform of COX and a long duration of action. It is classified chemically as a member of the sulphonamide subgroup of coxibs. Mavacoxib is highly lipid but very poorly water soluble. In the dog, the pharmacokinetic (PK) profile comprises very slow body clearance, long elimination half-life and a relatively large distribution volume. Biotransformation and renal excretion are very limited, and elimination occurs primarily by biliary secretion and excretion of unchanged drug in faeces. The PK profile of mavacoxib differs quantitatively between young healthy dogs (Beagles and mongrels) and clinical cases with osteoarthritis (OA). In OA dogs, mavacoxib exhibits a much longer terminal half-life, associated principally with their greater median body weight compared with dogs used in preclinical studies. There is also some evidence of breed differences and a small effect of age on mavacoxib PK in the OA canine population. The pharmacodynamics (PD) of mavacoxib has been established: (i) in whole blood assays at the molecular level (inhibition of COX-1 and COX-2 isoforms); (ii) in preclinical models of inflammation and pain; and (iii) in clinical OA subjects treated with mavacoxib. The dosage schedule of mavacoxib for clinical use has been determined by owner and veterinary clinical assessments and is supported by integration of PK and PD preclinical data with clinical responses in canine disease models and in dogs with naturally occurring OA. The dosage regimen has been further confirmed by correlating levels of inhibition of COX isoforms in *in vitro* whole blood assays with plasma concentrations of mavacoxib achieved in OA dogs. In addition to the specific properties of mavacoxib, some general aspects of the PK and PD of other agents of the NSAID group, together with pathophysiological and clinical aspects of OA, are reviewed, as a basis for correlating with the safety and efficacy of mavacoxib in therapeutic use. Integration of PK and PD data suggests that the recommended dosage regimen of 2 mg/kg bw once for 14 days, followed by administration at monthly intervals, is optimal from both efficacy and safety perspectives and is further confirmed by clinical field studies.

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## COMPARATIVE PHARMACOLOGY, TOXICOLOGY AND THERAPEUTICS OF MAVACOXIB

Only limited comparison of mavacoxib with other agents of the nonsteroidal anti-inflammatory drug (NSAID) class is possible in this review. The reader is referred to Berg and Budsberg (2005), Lascelles *et al.* (2005), Papich (2008), Lees (2009), KuKanich *et al.* (2012) and Monteiro-Steagall *et al.* (2013) for comparative reviews on NSAIDs in the dog.

## CHEMICAL STRUCTURE AND PHYSICO-CHEMICAL PROPERTIES OF MAVACOXIB

Mavacoxib is a NSAID of the coxib subgroup. Chemically, it is a substituted sulphonamide, structurally similar to celecoxib (Penning *et al.*, 1997) and differing only in the substitution of a methyl group by a fluorine atom (Fig. 1). The chemical name is 4-[5-(4-fluorophenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl]-benzenesulfonamide. There are five crystalline forms of mavacoxib (I–V), distinguished by powder X-ray diffraction. Form I is used in the commercial product; it is an anhydrous, nonsolvated and nonhygroscopic form. Mavacoxib is chemically and physically stable under ambient conditions. It is a weak organic base; the pKa is 9.57. Its physico-chemical properties include very low water solubility at room temperature (6 μg/mL) and very high lipid solubility. The XLOGP3 value is 3.1. According to Biopharmaceutics Classification System (Amidon *et al.*, 1995; Martinez *et al.*, 2002; Yu *et al.*, 2002), mavacoxib would likely be classified as a Class II compound, that is a poorly water soluble but highly permeable drug. It is therefore expected that mavacoxib in solution will be readily absorbed from the gastrointestinal tract (g.i.t.). The incomplete bioavailability reported in some trials (*vide infra*) is probably due to incomplete solubility, relating to food availability at the time of dosing. This is also relevant to enterohepatic recycling, as a study using radio-labelled mavacoxib demonstrated that 55% of parent mavacoxib can be detected in bile.

## CLINICAL INDICATIONS AND DOSAGE

Mavacoxib is indicated for the treatment of pain and inflammation in dogs with osteoarthritis (OA), aged 12 months or older (EMA, 2008). Commercially, mavacoxib is available as chewable tablets in five strengths, ranging from 6 to 95 mg per tablet. It is contraindicated in dogs weighing <5 kg. The manufacturer's recommended dosage is 2 mg/kg, administered with an interval of 14 days between doses 1 and 2 and intervals of 28 days for subsequent doses. The maximum duration of treatment is 6.5 months.

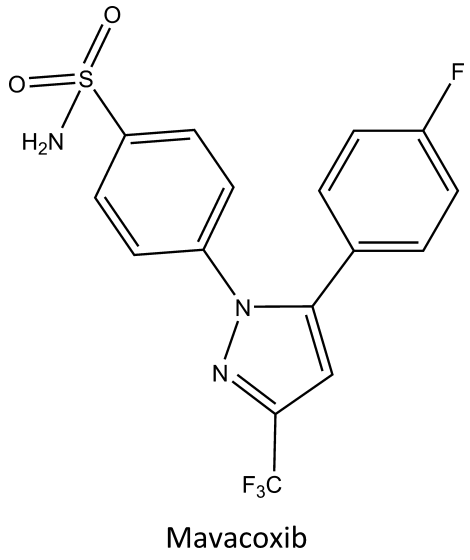


**Fig. 1.** Chemical structure of mavacoxib.

## PRECLINICAL AND POPULATION PHARMACOKINETICS

### *Preclinical pharmacokinetics in Beagle and mongrel dogs*

The pharmacokinetic (PK) profile of mavacoxib in Beagle dogs was described by Cox *et al.* (2010). Body clearance was very slow (0.045 mL/kg/min) and the volume of distribution rather large (1.64 L/kg), explaining the long elimination half-life of 17.3 days (Table 1). Across three groups of dogs administered mavacoxib (intravenous fasted, oral fasted and oral fed), the range of individual half-life was 9.6–38.6 days. Bioavailability was significantly greater at 87% in fed dogs compared with 46% in fasted animals (Table 1). Increased bioavailability in fed compared with fasted dogs has also been reported for other sulphonamide coxibs, including celecoxib (Paulson *et al.*, 2001) and deracoxib (Novartis Animal Health, 2007). These drugs have the common properties of low water and high lipid solubility, thus belonging to Class II (poorly soluble, highly permeable) of the Biopharmaceutics Classification System (Martinez *et al.*, 2002).

In addition to high bioavailability of mavacoxib in fed dogs, two further aspects of the absorption pattern are of interest. *First*, there was rapid attainment of a plasma concentration of 0.4 μg/mL, shown to provide a good degree of analgesia in preclinical models (*vide infra*). The mean concentration in fed dogs 1 h after dosing was 2.1 μg/mL and this was 80% of $C_{max}$. The data further suggest that, based on trough concentrations of

**Table 1.** Pharmacokinetics of mavacoxib in Beagle dogs (LS mean and 95% confidence intervals, $n = 10$)*

| Variable (units) | Route | LS mean (CI) |
|---|---|---|
| Cl (mL/kg/min)† | IV | 0.045 (0.035, 0.055) |
| $t_{½}$ (days) (fasted dogs) | IV | 17.3 (15.0, 20.5) |
| $t_{½}$ (days) (fed dogs) | Oral | 15.5 (13.6, 18.0) |
| $t_{½}$ (days) (fasted dogs) | Oral | 19.3 (16.5, 23.4) |
| $V_{d_{ss}}$ (L/kg) | IV | 1.64 (1.41, 1.87) |
| Plasma protein binding (%) | IV | >98 |
| $T_{max}$ (h) (fasted dogs) | IV | 0.55 (−25.3, 26.4) |
| $T_{max}$ (h) (fed dogs) | Oral | 17.4 (−8.4, 43.2) |
| $T_{max}$ (h) (fasted dogs) | Oral | 67.4 (41.6, 93.2) |
| $F$ (%) (fed dogs) | Oral | 87 (64, 120) |
| $F$ (%) (fasted dogs) | Oral | 46 (34, 63) |

Cl, body clearance; $t_{½}$, elimination half-life; $V_{d_{ss}}$, volume of distribution at steady-state; $T_{max}$, time of maximum concentration; $F$, bioavailability. *Data from Cox *et al.* (2010). †Clearance value can be compared with the value of 5.8 mL/kg/min defined by Toutain and Bousquet-Mélou (2004a) as a 'low' value for dogs weighing 10–20 kg. This indicates the very low clearance rate of mavacoxib.

mavacoxib, effective analgesia would be provided for the dogs in this PK study for 28 days (oral fed) and 21 days (oral fasted). In addition, in both fed and fasted orally dosed dogs and also after intravenous dosing, there were multiple early peaks in plasma mavacoxib concentration. Causes are unknown, but a possible partial explanation could be *repeated* enterohepatic recycling.

Cox *et al.* (2010) also described the PK of multiple oral doses (4 mg/kg) of mavacoxib in Beagle dogs, administered on days 0, 14, 42 and 70. Although $C_{max}$ was lower after dose 1, trough and average concentrations of mavacoxib were similar for all four doses (Table 2), indicating achievement of steady-state PK after the second dose. Concentrations predicted to provide good analgesic responses (i.e., >0.4 μg/mL), *vide infra* were provided by trough concentrations after all four doses.

Cox *et al.* (2010) reported on dose proportionality of the PK of mavacoxib in Beagle dogs with single oral doses of 2, 4 and 12 mg/kg. This was demonstrated for AUC and $C_{max}$. They also described dose proportionality in mongrel dogs in a multiple dose (5, 15 and 25 mg/kg), multiple dosing (days 0, 14, 42, 70, 98, 126 and 154 days) study. To maximize systemic drug exposure, each dog was allowed access to food within 1–2 h after each dose. Dose proportionality for maximum and average concentrations and also $AUC_\tau$ was demonstrated across the dosage range of 5–25 mg/kg for both first and final administered doses. The dose normalized steady-state $AUC_\tau$ values in this study were similar to $AUC_\infty$ values determined in fed dogs in the single dose absolute bioavailability study. Therefore, the data suggest that the PK profile of orally administered mavacoxib is similar in young Beagles and young mongrels of similar body weights.

### *Plasma protein binding*

The binding to plasma protein of mavacoxib exceeds 98% of total concentration (Cox *et al.*, 2010). Assuming linear binding as demonstrated by Cox *et al.* (2010), the total and free concentrations are proportional to an unbound (fu) factor and the same maintenance dose can be accurately computed, based either on the total or on the free plasma clearance (Toutain and Bousquet-Mélou (2004a), thus:

$$\text{Maintenance monthly dose} = \text{Monthly Plasma (total) clearance} \times \text{Target (total) concentration}$$

or

$$\text{Maintenance monthly dose} = \text{Monthly Plasma (free) clearance} \times \text{Target (free) concentration.}$$

**Table 2.** Maximum and trough concentrations of mavacoxib over the dosing interval in Beagle dogs after multiple oral doses at a dosage of 4 mg/kg (LS mean and 95% confidence intervals, $n = 9$)*

| Dose number (dosing study day) | $C_{max}$ [LS mean (CI)] (μg/mL) | $C_{trough}$ [LS mean (CI)] (μg/mL) |
|---|---|---|
| 1 (1) | 1.66 (1.20, 2.28) | 0.96 (0.72, 1.28) |
| 2 (14) | 3.32 (2.70, 4.08) | 0.89 (0.67, 1.18) |
| 3 (42) | 2.86 (2.15, 3.82) | 0.83 (0.62, 1.10) |
| 4 (70) | 2.71 (2.06, 3.58) | 0.77 (0.60, 0.99) |

Data from Cox *et al.* (2010). *Dosing interval was 14 days between first and second doses and 28 days between subsequent doses.

### *Population pharmacokinetics in osteoarthritic dogs*

Martinez and Modric (2010) have pointed out that 'when PK data are generated in small groups of normal healthy animals, it is often assumed that these data represent the drug's PK characteristics across the intended patient population'. The statement of Martinez and Modric highlights the importance to extrapolate PK data derived from young healthy animals to older and possibly diseased populations of animals with great caution. The population inferential value of those data is rarely considered. Cox *et al.* (2011) described population PK data for mavacoxib in two field trials, incorporating primarily elderly (median age = 10 years) large-breed dogs, all diagnosed with OA. In both trials, dogs received the commercial tablet formulation at dosage of 4 mg/kg for seven doses (trial 1) and 2 mg/kg for five doses (trial 2). The dosing interval was 2 weeks between first and second doses, then monthly thereafter and up to seven doses per animal were administered. Mavacoxib trough concentrations in plasma were determined on a total of 1317 samples from 286 dogs. The population PK analysis was undertaken using the nonlinear mixed effect modelling programme NONMEM v. 6.1.0. (ICON, Hanover, MD, USA), incorporating various subject demographic variables, including age, sex, breed and weight. The contribution of each explanatory variable was 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 predictive performance of the final model was evaluated by Monte Carlo simulations, and a large majority of observations were fitted well by the selected model.

In the final model, clearance (Cl) and volume of distribution ($V_d$), each scaled by bioavailability ($F$), were as follows: $Cl/F$ = 1.35 L/day (0.039 L/day/kg) with between subject variability (BSV) = 46.9%; $V_d/F$ = 85.7 L (2.45 L/kg) with BSV = 19.4%. Body weight was the primary factor predicting both variables, but the model also predicted smaller effects of age and breed on $Cl/F$ (but not $V_d/F$). The model for a typical dog, weighing 35 kg and 10 years old, predicted values of $Cl/F$ and $V_d/F$, which were power functions of body weight with coefficients of 0.787 and 0.981, respectively. Thus, $Cl/F = 1.35 \times (WT/35)^{0.787} \times (Age/10)^{-0.215} \times (1 + 0.314 \times Breed)$ L/day, where breed is an indicator variable with a value of 1 for Labrador retrievers or German shepherds, but 0 otherwise and $V_d/F = 85.7 \times (WT/35)^{0.981}$.

The mean terminal half-life ($t_{½}$) determined from empirical Bayes estimates for 286 dogs was 44 days and $t_{½}$ increased

with body weight, but only slightly. The population PK model was in agreement with data collected in Beagles and mongrels in preclinical PK studies. Thus, the population PK model predicted a $t_{½}$ for a typical laboratory dog (1-year-old 10-kg body weight) a $t_{½}$ of 21 days, which is in agreement with the actual finding of 17 days. The mavacoxib $t_{½}$ in the typical OA dog (BW = 35 kg, 10 years old) was 44 days, the greater value being accounted for mainly by the weight but also by the age difference. A typical dog was predicted to attain steady-state plasma concentrations after 4–7 months. However, 4.6% of the dogs had long half-lives, ranging from 80 to 140 days. In these dogs, trough concentrations of mavacoxib increased with each administered dose, so that steady-state was not achieved within the 6.5 month long study. Prolonged $t_{½}$ was not associated with any covariate factor. Cox *et al.* (2011) postulated that a polymorphism of a transporter involved in the biliary clearance of mavacoxib might account for and be predictive of prolonged $t_{½}$ in a small proportion of geriatric large-breed OA dogs.

### *Determination of an upper bound for maintenance dose for mavacoxib in OA dogs*

The selection of a maintenance dose for NSAIDs is a difficult task and, at first consideration, the optimal dose is the highest possible dose that is safe for all dogs. For NSAIDs, it is commonly reported that the plasma concentration for which COX-1 inhibition is <20% (i.e., the $IC_{20}$) is a safe plasma concentration for g.i.t toxicity (*vide infra*); for mavacoxib, the $IC_{20}$ for COX-1 from a whole blood assay was 2.46 μg/mL. The corresponding upper limit for a maintenance dose administered at 28 day intervals (the selected clinical dosing interval) can be estimated by solving the population equation Cl/F, CL/F being the only PK parameter controlling internal drug exposure. Considering dogs aged 10 years and of 10, 20 and 40 kg BW, the upper bounds of a safe maintenance dose were estimated to be 3.47, 2.99 and 2.58 mg/kg, respectively, for all canine breeds. For Labradors and German Shepherds, however, the predicted upper bound for the maintenance dose is 1.314-fold higher than for all breeds. Overall, it is suggested that a 2 mg/kg BW dose, administered at 28-day intervals, is a likely safe dose for most dogs (see section PKs and adverse events). For a dose of 2 mg/kg for 10-year-old dogs, predicted average plasma concentrations over the dosing interval are 1.41, 1.64 and 1.90 μg/mL in dogs weighing 10, 20 and 40 kg, respectively, and they are smaller by a factor of 1.314 for Labrador and German Shepherd breeds.

### *Rationale for determination of a dosing interval for mavacoxib in osteoarthritic dogs*

The therapeutic index, which is typically considered as the ratio of the highest exposure to the drug that results in no toxicity to the exposure that produces the desired effect (Muller & Milton, 2012) can be established using the second component of a dosage regimen, that is the dosing interval. Establishing an appropriate dosing interval is essential to ensure that plasma mavacoxib concentrations fluctuate only within its therapeutic window, that is within the range of plasma concentrations associated with safety and efficacy. The therapeutic window is delimited by two critical concentrations: a lower concentration below which the probability of achieving adequate efficacy is too low and an upper concentration above which the risk of side effects occurring outweighs the potential benefit from any additional therapeutic effect (Rowland & Tozer, 1995). For most NSAIDS, the therapeutic window is considered to be rather narrow, although this varies between drugs and variation occurs for individual animals also. The limits of upper and lower concentration are not known with precision for any NSAID including mavacoxib but, as a general rule, Rowland and Tozer (1995) recommend for this drug class that the upper and lower limits differ by a factor of no more than 2 or 3. The plasma concentration fluctuations between $C_{max}$ and $C_{min}$ can be predicted from the dosing interval (a decision for the clinician) and the plasma half-life (a drug property; see also Toutain & Bousquet-Mélou, 2004b for details).

The dosing interval should be selected to ensure that plasma concentration fluctuates minimally from the efficacious steady-state plasma concentration, while the dosing interval must be compatible with the owner's routine to guarantee dosing compliance (Lees & Maddison, 2006). When dosing interval is small relative to half-life, the amplitude of fluctuation in plasma concentration will likewise be small.

For an individual dog with a dosing interval of 28 days and a $t_{½}$ also of 28 days, it can be demonstrated that $C_{max}/C_{min} = 2$. In the case of an animal with $t_{½} = 78$ days and $\tau = 28$ days, $R = 4.54$ and $C_{max}/C_{min} = 1.28$ under steady-state conditions. Therefore, for only a minority of dogs in the clinical population (with $t_{½} < 28$ days) is the $C_{max}/C_{min}$ ratio predicted to yield a value >2.

The actual dose interval selected of 28 days therefore yields a $C_{ss,\ max}/C_{ss,\ min}$ ratio of the order of 1.6, which may be regarded as acceptably small from both efficacy and safety perspectives. These calculations apply for any maintenance dose (actually 2 mg/kg for mavacoxib), a fixed dosing interval of 28 days, and a clinical population derived *mean* $t_{½}$ of 40 days.

From the population parameters, the steady-state $C_{max}$ and $C_{min}$ can be also computed; for a typical OA dog of 35 kg BW, for a maintenance dose of 2 mg/kg every 28 days and an half-life of 40 days, the predicted $C_{ss,\ max}$ is 2.08 μg/mL and the predicted $C_{ss,\ min}$ is 1.28 μg/mL giving the expected $C_{ss,\ min}/C_{ss,\ min}$ ratio of 1.6 for the therapeutic window. It is interesting to note that this computed minimal plasma concentration in steady-state condition is equal to the $IC_{80}$ of COX-2 inhibition, as determined from a whole blood assay (*vide infra*, Table 3), suggesting that trough concentrations of mavacoxib significantly inhibit COX-2.

### *Metabolism and excretion*

The metabolism and excretion patterns of mavacoxib administered intravenously or orally have been established in $^{14}$C-radiolabel studies in Beagle dogs (M. Stegemann, unpub-

**Table 3.** Potency of mavacoxib and potency ratios of mavacoxib and carprofen in *in vitro* canine whole blood assays

| Magnitude of inhibition | Mavacoxib inhibition of COX-1 (μg/mL)* | Mavacoxib inhibition of COX-2 (μg/mL)† |
|---|---|---|
| $IC_{20}$ | 2.46 | 0.169 |
| $IC_{50}$ | 8.73 | 0.394 |
| $IC_{80}$ | 48.44 | 1.28 |

| | Potency ratios COX-1:COX-2 | |
|---|---|---|
| | Mavacoxib | Carprofen |
| $IC_{20}$COX-1:$IC_{20}$COX-2 | 14.5:1 | 10.9:1 |
| $IC_{50}$COX-1:$IC_{50}$COX-2 | 22.1:1 | 17.2:1 |
| $IC_{80}$COX-1:$IC_{80}$COX-2 | 37.8:1 | 30.8:1 |
| $IC_{20}$COX-1:$IC_{80}$COX-2 | 1.92:1 | 1.95:1 |

For mavacoxib, all differences between COX-1 and COX-2 were significant ($P < 0.0001$). Potency ratio differences between carprofen and mavacoxib were nonsignificant. *Assay based on blood allowed to clot for 45 min under standard conditions. †Assay based on induction and activation of COX-2 by *Escherichia coli* derived lipopolysaccharide with incubation time of 21 h.

lished data). For plasma concentration, mavacoxib parent compound accounted for at least 90% of total concentration at all times and at least 95% 72 h after dosing. Expressed as a percentage of orally administered dose, the daily excretion of total radio-labelled residue was of the order of 0.2–0.4% in urine and 0.7–1.4% in faeces. In faeces, most of the residue (>58%) was parent drug; in urine, most was metabolites (≤9% of parent drug). The principal elimination mechanism is secretion in bile. In this respect, the comment of Treinen-Moslen and Kantz (2006) that 'acyl glucuronides are plausible proximate toxicants for the small intestinal injury caused by NSAID, based on their reactivity and extent of secretion into bile', may be noted. Obviously, direct effects cause by the parent molecule can also occur albeit using different mechanisms. However, for mavacoxib, no acyl glucuronide, that is an electrophilic metabolite with sufficient reactivity to adduct proteins and other biomolecules, was detected in urine, faeces or bile. This is relevant to the safety of mavacoxib in relation to any potential enteropathic effects (*vide infra*).

## PHARMACODYNAMICS

### *Inhibition of cyclooxygenases as the principal mechanism of action of NSAIDs*

For COX inhibitors, COX-2 inhibition is considered by most authors to be the molecular action most related to the therapeutically required anti-inflammatory effect, although some authors have postulated an additional role for COX-1 (Wallace *et al.*, 1998). Most authors have attributed inhibition of COX-1 to the adverse effects, in relation to perforation, ulceration and bleeding in the g.i.t. and inhibition of blood clotting pathways. Nevertheless, it has been argued that both COX-1 and COX-2 contribute to gastric mucosal defence (Wallace, 2008). Indeed, it is recognized that 'inhibition of COX-1 bad and inhibition of COX-2 good' is an oversimplification of a much more complex situation. For example, some experimental data indicate that COX-1 selective inhibitors, as well as the newer drugs producing selective inhibition of COX-2, have lower ulcerogenic potential than nonselective inhibitors (Wallace, 2008). Moreover, rodent and canine studies have shown that COX-2 selective inhibitors may delay the healing of stomach ulcers (Wallace, 2008; Goodman *et al.*, 2009). This may be relevant to the safety of COX-2 inhibitors, in the light of the report of Wooten *et al.* (2010) that dogs that appear to be clinically normal may have underlying g.i.t. lesions associated with upregulation of COX-2. On the other hand, Fornai *et al.* (2014), in a study of small bowel integrity in rats, suggested that nonselective NSAIDs and etoricoxib can induce enteropathy through a topical action, whereas celecoxib lacked similar detrimental actions. The selectivity profile of COX-1/COX-2 inhibition by test drugs and the related effects on prostaglandin production did not appear to play a major role in the pathogenesis of enteropathy.

It is possible that selective/preferential COX-2 inhibitors at recommended dosage might exacerbate hypercoagulable states. However, laboratory data in healthy Beagle dogs demonstrate that a dose of 10 mg mavacoxib/kg BW (5× the label dose) had no effect on platelet function as determined by buccal mucosal bleeding time 16 days after administration (Krautmann *et al.*,2009). Moreover, the precise consequences of COX-1 and COX-2 inhibition in the canine kidney have yet to be resolved (Papich, 2008; KuKanich *et al.*, 2012). It is clear that there are species differences in terms of the extent and distribution of COX-2 expression in the kidney. For example, in the dog and rat, the macula densa expresses COX-2 and volume depletion leads to marked upregulation of this protein whereas in the monkey and in human, no COX-2 expression can be detected (Khan *et al.*, 1998) even on volume depletion of monkeys. In the dog, chronic treatment with furosemide and COX-2 preferential inhibitors (carprofen and etodolac) led to a reversible reduction in GFR (Surdyk *et al.*, 2012), suggesting that COX-2 plays a role in sustaining GFR in volume depleted dogs, although the effect of nonselective COX inhibitors in this setting has not been studied.

Nevertheless, for efficacy the greater the amount of time within the interdosing interval that COX-2 can be substantially inhibited (*vide infra*), while COX-1 is relatively unaffected, the safer and more effective the dosage regimen is likely to be, at least for g.i.t. toxicity, for healthy tissue and inhibition of blood clotting (Mitchell & Warner, 1999; Warner *et al.*, 1999; Lees *et al.*, 2004).

Cyclooxygenase-2 is formed in response to tissue injury, irrespective of cause (Seibert *et al.*, 1994; Crofford, 1997; Zhang *et al.*, 1997; Claria, 2003). The initial expectation was that selective COX-2 inhibitors would be free of the side effects commonly associated with classical NSAIDs, which generally are nonselective COX inhibitors (Masferrer *et al.*, 1994). However, much subsequent research has identified COX-2 constitutively in several organs, including the spinal cord, bone, joints, eye,

kidney, pyloric and duodenal mucosa and vascular endothelial cells (Flower, 2003; Papich, 2008; Wooten *et al.*, 2008, 2009). The precise roles of constitutively expressed COX-2 in tissues are the subject of ongoing research (Flower, 2003). Nevertheless, the general finding from many preclinical and clinical studies in several species has been that COX-2 selective drugs have represented a significant therapeutic advance from a g.i.t. safety perspective (KuKanich *et al.*, 2012). While it has to be acknowledged that controlled studies in dogs comparing the gastrointestinal safety of COX-2 selective with nonselective NSAIDs are not available, it is generally accepted that KuKanich's observations extrapolate to the situation in dogs as well.

All coxibs are, to varying degrees, selective or preferential COX-2 inhibitors. For mavacoxib, the basis for the selectivity is indicated in Fig. 2, which illustrates diagrammatically the steric hindrance, arising from its molecular conformation. This limits entry through the channel of access to the acid recognition and acetylation sites of the COX-1 molecule. For comparative purposes, the entry of ketoprofen, a nonselective COX inhibitor in the dog, into both sites, is also illustrated.

*In vitro* and *ex vivo* whole blood assays have been developed by many groups to determine concentration–response relationships of NSAIDs for COX-1 and COX-2 inhibition (Warner *et al.*, 1999; Brideau *et al.*, 2001; Giraudel *et al.*, 2005a, 2009). This has allowed the following: (i) potency determination expressed as percentage degree of inhibition, usually 50% ($IC_{50}$), but also other levels of inhibition (e.g., $IC_{20}$, $IC_{80}$, $IC_{95}$); and (ii) determination of potency ratios for the two COX isoforms, usually expressed as $IC_{50}$COX-1:$IC_{50}$COX-2. However, some groups have preferred consideration of 80–50% inhibition ratios, because this level of inhibition of COX-2 is required to ensure good clinical control of pain (Warner *et al.*, 1999; Lees *et al.*, 2004; Giraudel *et al.*, 2005a,b, 2009). Of relevance clinically, with respect to the absence of, or minimal effects on, clotting pathways and the g.i.t. and therefore to safety, is the magnitude and time course of inhibition of COX-1 throughout the interdose interval (Lanza *et al.*, 1999).

A precise percentage inhibition of COX-1 for all NSAIDs, which should not be exceeded on safety grounds, for most or the entire interdose interval, cannot be stated with certainty. The ideal might be no inhibition but Lees *et al.* (2004) and Giraudel *et al.* (2005a) have suggested a value of 20%. Based on these considerations, the latter group has proposed estimation of the $IC_{20}$COX-1:$IC_{80}$COX-2 ratio as a useful but indirect and approximate indicator of the toxicity: efficacy ratio of NSAIDs for the g.i.t. However, a consideration particularly relevant to mavacoxib is that the peak concentration occurs only once every 28 days shortly after dosing. Whether this infrequent peak together with postpeak concentrations will provide (other things being equal), a similar safety profile as a peak concentration that occurs once daily for a drug with shorter half-life is an interesting but unanswered question.

### *Peripheral and central cyclooxygenase-2 as a target for action*

There are no published data on mavacoxib to indicate its principal site of action (peripheral or central) in the dog. However, there is circumstantial evidence that mavacoxib provides therapeutic benefit through spinal actions, as well as actions at

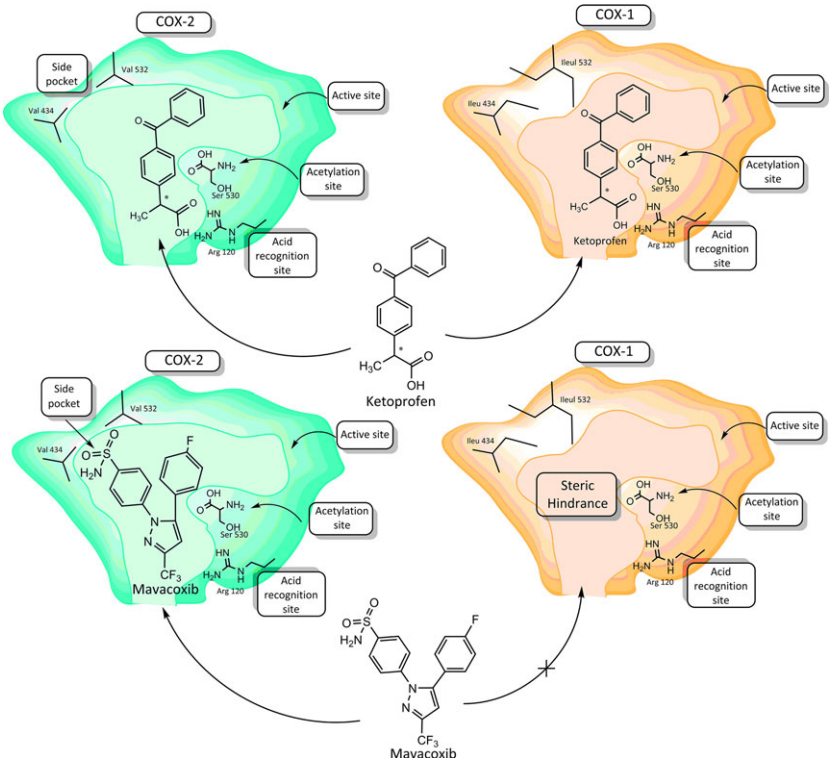


**Fig. 2.** Diagram illustrating access to active sites of COX-1 and COX-2 by ketoprofen, a nonelective inhibitor of COX-1 and COX-2 in the dog, and mavacoxib, a preferential selective COX-2 inhibitor in the dog.

peripheral sites of inflammation. Although mavacoxib is highly bound to proteins, it can easily dissociate, permeate through the blood–brain barrier and thus be available to any action at the spinal level (Tanak & Mizojiri, 1999). Moreover, a role for spinal cord mediated sensitization in the pain of human OA patients is now widely accepted (Imamura *et al.*, 2008; Read & Dray, 2008).

COX-2 is expressed constitutively in dorsal horn cells of the spinal cord and is also induced peripherally at inflammatory sites in response to tissue damage (Kujubu *et al.*, 1991; Xie *et al.*, 1991; Crofford *et al.*, 1994; Seibert *et al.*, 1994; Crofford, 1997). The continuous production of pro-inflammatory prostaglandins, for example PGE2 by COX-2 peripherally, and probably centrally also, is believed to be a critical element in maintaining hyperalgesic responses (Zhang *et al.*, 1997). At the local level, the source of PGE2 may be infiltrating leucocytes or resident tissue cells (Masferrer *et al.*, 1994). Dirig *et al.* (1998) proposed that spinal COX-2 was required for the initiation of thermal hyperalgesia, whereas peripheral COX-2 was important in maintaining hyperalgesia associated with tissue injury.

The expression of COX in synovial tissues of human patients with rheumatoid arthritis (RA) and (to a lesser degree) OA was demonstrated in early studies by Sano *et al.* (1992). Others demonstrated the selective upregulation of COX-2 mRNA and COX-2 protein in adjuvant induced arthritis in the rat, soon after discovery of COX-2 (Anderson *et al.*, 1996). The latter authors reported inhibition of PGE2 synthesis in this model by a selective COX-2 inhibitor, accompanied by decreased synovial inflammation. Koki *et al.* (2002) reported prominent expression of COX-2 in human OA knee joints, not only in the synovium but also in blood vessels and the fibrocartilage of osteophytes. Amin *et al.* (1997) reported superinduction of COX-2 in human OA cartilage. Likewise, COX-2 upregulation occurs in canine OA of the hip joint in the synovium, joint capsule and subchondral bone (Lascelles *et al.*, 2009). Martel-Pelletier *et al.* (2003) reviewed the extensive literature in this field and confirmed the upregulation of COX-2 leading to synthesis of PGE2 in articular chondrocytes and synovial fibroblasts. Laboratory animal and tissue culture studies have been supported by reports on the efficacy of coxibs in human OA and RA patients (Martel-Pelletier *et al.*, 2003).

There is also abundant evidence of upregulation of COX-2 in the spinal cord in response to peripheral inflammation. COX-2 is present constitutively in the spinal cord, and COX-2 mRNA is also induced in adjuvant induced arthritis in the rat (Beiche *et al.*, 1996; Hay *et al.*, 1997), indicating a significant role in the development of hyperalgesia and allodynia (Schaible *et al.*, 2006). Dolan *et al.* (2003) reported increased message and COX-2 protein in lamina V dorsal horn neurones of sheep 1 day after surgical inflammation (laparoscopy). Neugebauer and Schaible (1990) described a role for the sensitization of spinal neurones in acute arthritis in the cat, while Sluka *et al.* (1994a) reported reduction of joint inflammation by dorsal rhizotomy. Samad *et al.* (2001) and Veiga *et al.* (2004) described (i) the pain hypersensitivity which arises in neighbouring uninjured tissue (secondary hyperalgesia) caused by increased neuronal activity in the spinal cord and (ii) a syndrome comprising diffuse muscle and joint pain, lethargy and anorexia. These responses were attributable to widespread induction of COX-2 in the spinal cord. Suppression of both joint inflammation and hyperalgesia by blockade of central sensitization pathways was also demonstrated by Sluka *et al.* (1994b). These authors reported reduced joint swelling and decreased hyperalgesia in kaolin and carrageenan-induced models of arthritis, supporting a reduction in rate of disease progression following blockade of COX-2 in the spinal cord (Imamura *et al.*, 2008; Read & Dray, 2008).

### *Comparison of mavacoxib and carprofen in in vitro COX-1 and COX-2 assays*

From the data in Table 3, it will be seen that potency ratios, expressed at three levels ($IC_{20}$, $IC_{50}$ and $IC_{80}$), for COX-1 and COX-2 were similar for mavacoxib and carprofen as a control drug (Lees *et al.*, 2009). For both drugs, inhibitory ratios were smallest for $IC_{20}$s and greatest for $IC_{80}$s; these differences are attributable to a similar lack of parallelism of the COX-1 and COX-2 inhibition curves for the two drugs. Based on these data, mavacoxib and carprofen can probably be classified as borderline between preferential and selective for COX-2 inhibition. It should be noted, however, that no single numerical value for inhibition ratios can be assigned to distinguish between preferential and selective action for COX-2, as this depends on slopes of inhibition curves. Moreover, plasma concentrations achieved *in vivo* with recommended dosage of NSAIDs may be associated with inhibition of both isoforms, even with preferential inhibitors (Lees *et al.*, 2004; Giraudel *et al.*, 2005a). As shown above, a recommended dose of mavacoxib of 2 mg/kg at 28-day intervals allows maintenance of plasma trough concentration less than the $IC_{20}$ for COX-1 inhibition.

The indicator in healthy animals of g.i.t. toxicity relative to efficacy proposed by Giraudel *et al.* (2005a, 2009), $IC_{20}$COX-1: $IC_{80}$COX-2, is of interest. It was, for mavacoxib and carprofen, almost identical, 1.92:1 and 1.95:1, respectively (Table 3, Lees *et al.*, 2009). This suggests the likelihood of a broadly similar level of safety for the g.i.t. relative to efficacy for the two drugs. However, this would apply only if doses used clinically in the dog provided plasma concentrations exerting similar magnitudes of inhibition of COX isoforms for the two drugs. Moreover, the comparison can provide only a reasonable approximation to toxicity: efficacy ratios for two further reasons. First, plasma concentrations will be subject to greater within day variability for carprofen. For this drug, the PK profile and dosage regimen dictate daily dosing, leading to peaks and troughs of concentration during the 24-h dosing interval. Such daily variations are slight for mavacoxib, the concentration of which declines very slowly between the long 14-/28-day dosing intervals. Secondly, direct exposure from oral dosing of g.i.t. mucosal cells occurs at 28-day intervals after the second dose of mavacoxib, whereas for carprofen and other NSAIDs, the local exposure occurs once or twice daily. However, daily exposure of the g.i.t. mucosa to mavacoxib will

occur due to its biliary excretion. If one assumes that all mavacoxib is excreted via bile (i.e. worst case scenario), the mavacoxib 'dose' exposed daily to the g.i.t. mucosa will be based on plasma clearance and peak/trough concentrations (see Table 2) as follows:

$$\text{Maximal Daily amount of mavacoxib excreted by the bile} = \text{daily plasma clearance} \times C_{max}$$

or

$$\text{Minimal Daily amount of mavacoxib excreted by the bile} = \text{daily plasma clearance} \times C_{min}$$

These equations can be solved with a daily plasma clearance of 0.045 mL/min/kg × 1440 min (Table 1) and (i) a maximal plasma concentration of approximately 3 μg/mL and (ii) minimal plasma concentration of approximately 1 μg/mL for a dose of 4 mg/kg (Table 2). It is computed that (i) the maximal amount of mavacoxib excreted daily by the bile is <200 μg/kg, that is <10% of the monthly dose; and (ii) in the 24 h immediately before the next administration of mavacoxib (at trough concentration), the amount of mavacoxib eliminated by the bile is equivalent to approximately 1.5% of the recommended maintenance dose. As only a small amount of mavacoxib is eliminated in the bile and intestinal exposure is predicted to be low, it can be hypothesised that this may produce better tolerability than for other NSAIDs such as carprofen that are administered orally once daily, although currently there is no evidence of that.

Observations within a large field safety and efficacy study seem to support the better gastrointestinal tolerability of monthly dosing compared with daily dosing; 195 of 1303 (15.0%) mavacoxib-treated dogs compared with 338 of 1295 carprofen-treated dogs (26%) exhibited digestive tract disorders (Six *et al.*, 2012). However, it should be noted that in this unmasked study, the higher number of adverse events reported for carprofen could be biased by the heightened owner's awareness to report gastrointestinal effects with a daily tablet administration.

### *Pharmacokinetics and adverse events*

While the PK of mavacoxib provides a number of advantages in the treatment of chronic OA, including potential higher compliance, it has to be acknowledged that the inability to terminate exposure with mavacoxib might be perceived as an increased safety risk. A series of studies were conducted to either alter enterohepatic recirculation or increase the rate of metabolic clearance of mavacoxib. The results showed that activated charcoal, cholestyramine, rifampin nor ursodiol had no effect (M. Stegemann, personal communication). However, within both pivotal registration and postmarketing studies, no clinically relevant differences were observed between the safety profile of monthly mavacoxib treatment compared with daily carprofen treatment, when dogs were treated for up to 6.5 months (Payne-Johnson *et al.*, 2009a,b, 2014; Six *et al.*, 2012).

Within a large postmarketing study ($n$ = 2598 dogs), a total of 595 mavacoxib-treated and 568 carprofen-treated dogs remained on study for 194 days. The following observations were made (Six *et al.*, 2012). The mean time (days) to onset of abnormal clinical signs was 80 and 76 for mavacoxib and carprofen, respectively. The most commonly observed abnormal clinical signs were digestive tract disorders (21%, 195 mavacoxib; 338 carprofen), systemic disorders (15%, 188 mavacoxib; 213 carprofen), and skin and appendage disorders (11%, 162 mavacoxib; 118 carprofen). The median duration of observed clinical signs associated with diarrhoea was 3 and 2 days for mavacoxib- and carprofen-treated dogs, respectively. Distribution (%, $n$) of adverse event seriousness was as follows: nonserious (75%, 391 mavacoxib, 516 carprofen), lack of efficacy (13%; 72 mavacoxib, 81 carprofen) and serious (12%; 72 mavacoxib, 78 carprofen).

Despite the fact that the effects of treatment with mavacoxib cannot be terminated, field comparative data showed that the safety profile of monthly mavacoxib and daily carprofen is similar. The similarity in both frequency and nature of adverse events observed after monthly (mavacoxib) and daily (carprofen) dosing suggests that in many cases, supportive therapy restores fluid balance and renal/g.i.t, blood supply in a way that continued COX inhibition is no longer detrimental to the animal. In other animals, the adverse effect itself, and that the underlying disease that predisposed to it, is either sufficiently severe and has resulted in irreversible organ damage such that immediate cessation of drug therapy does not influence the negative outcome of the case, or is self-limiting such that supportive therapy is required for a period of time that is not related to the duration of action of the NSAID.

As with any other NSAID, experimental target animal safety studies do not usually detect renal toxicity because the animals used are young healthy animals that remain well hydrated throughout the dosing period. This was the case with mavacoxib at 15 mg/kg in a 6 month oral dosing study where no definitive biochemical evidence of renal damage was detected (M.J. Krautmann, J.F. Boucher. & M. Stegemann, personal communication). Furthermore, in the registration field study at the label dose of 2 mg/kg bw, the incidence of serious suspected adverse product experiences affecting the kidneys was very low for both mavacoxib and carprofen, when treatment was continuously administered for up to 6.5 months (Six *et al.*, 2012; Payne-Johnson *et al.*, 2014). Relatively short time to onset of these renal adverse events, as well as pathological findings, suggests that pre-existing renal pathology probably predisposed to the adverse events. Nevertheless, it is acknowledged that the clinical implications for renal health of administering NSAIDs chronically to aged dogs with OA are unknown and would require a comparison to placebo to control for the background rate of development and progression of azotaemic chronic kidney disease in this population of dogs. Such a study would not be ethical, as these dogs require some form of pain relief to improve their quality of life. Moreover, the relative

safety of all COX-2 preferential drugs and indeed nonselective COX inhibitors also, when administered chronically to dogs, remains to be determined. Constitutive expression of both COX-1 and COX-2 occurs in the canine kidney, suggesting that both enzymes play a role in kidney physiology. Supporting hydration and maintaining extracellular fluid volume and blood pressure are important measures that are advised to avoid acute kidney injury and a sudden fall in GFR in dogs receiving NSAIDs, and appropriate recommendations can be found on the product label of both daily and monthly administered coxibs.

*Pharmacodynamic–pharmacokinetic correlations in canine models with clinical endpoints*

For a typical OA dog, 35 kg in weight and having a mavacoxib half-life of 40 days, the predicted $C_{ss,max}$ is 2.08 μg/mL and the predicted $C_{ss,min}$ is 1.28 μg/mL. In this section, the effective/safe plasma concentrations actually obtained in *in vivo* studies conducted in the dog are considered. For this purpose, total plasma concentration is considered, as this is the driving concentration for all local concentrations, including the CNS local concentration.

Analgesic efficacy of mavacoxib in a carrageenan-induced metacarpal footpad lameness model was achieved with a plasma mavacoxib concentration of 0.454 μg/mL at day 22 after mavacoxib dosing for walking lameness. Significant efficacy for both walking and standing lameness was obtained at day 15, when plasma concentration was 0.766 μg/mL. It is reasonable to assume at least similar efficacy prior to 22 days (walking lameness) and before 15 days (both walking and standing lameness), with the higher plasma concentrations at the earlier times.

In an acute synovitis model, excellent analgesia was obtained 30 days after dosing, when mean mavacoxib plasma concentration was 0.411 μg/mL (Table 4, Lees *et al.*, 2009). The data from these footpad and synovitis models may be compared with plasma mavacoxib concentrations for $IC_{50}$ and $IC_{80}$ for COX-2 of 0.394 and 1.28 μg/mL, respectively. Thus, excellent analgesia was achieved 30 days after dosing in the synovitis model with a concentration approximately equal to the $IC_{50}$ for COX-2 (Tables 3 & 4). The mavacoxib dosage regimen of 2 mg/kg at 28-day intervals, selected for clinical use, provided, in a clinical study in OA dogs, trough concentrations of 0.52 μg/mL (14 days after dose 1) and 1.11 μg/mL (28 days after dose 5). Therefore, because of the differing PK profiles between the preclinical and clinical dogs, the trough concentrations in the OA clinical population exceeded those providing good efficacy in the synovitis model and also exceeded the $IC_{50}$ for COX-2 at all times.

*Integration of trough concentrations of mavacoxib in osteoarthritic dogs with in vitro assays of COX inhibition*

A NSAID dosage regimen that provides minimal risk for g.i.t. toxicity and inhibition of clotting side effects should preferably not exceed $IC_{20}$ for COX-1 inhibition for most if not all of the interdose interval, that is a concentration of the order of 2.46 μg/mL for mavacoxib.

Trough plasma concentrations of mavacoxib in the two clinical studies in OA dogs reported by Cox *et al.* (2011) are presented in Table 5. At the 2 mg/kg dosage, the maximum trough concentrations were obtained after the 5th dose (1.1 ± 0.50 μg/mL): maximum plasma concentrations were not reported in these trials but, using the 1.6 factor for the $C_{ss,max}/C_{ss,min}$ ratio as derived above, the corresponding maximum plasma concentration is predicted to be 1.76 ± 0.8 μg/mL. These values are consistent with those predicted for typical OA dogs of 35 kg BW and having a half-life of 40 days, with a $C_{ss,max}$ of 2.08 μg/mL and a predicted $C_{ss,min}$ of 1.28 μg/mL. In fact, in no dog at any sampling time did the trough plasma concentration exceed 2.46 μg/mL (the $IC_{20}$ for COX-1 inhibition) for a maintenance dose of 2 mg/kg. Moreover, based on computer simulation of plasma concentration in a dog with a $t½$ exceeding 80 days (and not attaining steady-state after five doses), receiving 2 mg/kg mavacoxib, the peak concentration after the fifth dose was <2.46 μg/mL.

In Study 1 of the population PK study of Cox *et al.* (2011) at 4 mg/kg mavacoxib dosage, the highest trough mean concentration of mavacoxib in plasma was 2.60 μg/mL after the seventh dose (Table 5), which is close to the $IC_{20}$ for COX-1 of 2.46 μg/mL (Table 3). Considering the 1.6 scaling factor, the

**Table 4.** Plasma mavacoxib concentrations and visual analogue scores (VAS) assessed at 3 h* in a canine acute synovitis model

| Treatment and dose ($n$ = 10/group) (mg/kg) | Plasma concentration† Mean ± SD (μg/mL) | VAS value† LSM (95% CI) | VAS difference from placebo | VAS statistics‡ $P$-value |
|---|---|---|---|---|
| 0 (placebo)[a] | 0 | 8.35 (6.60, 10.10) | – | – |
| 0.5[a] | 0.084 ± 0.053 | 6.74 (4.92, 8.56) | 1.61 | 0.116 |
| 1.0[a] | 0.114 ± 0.064 | 7.62 (5.87, 9.37) | 0.73 | 0.457 |
| 2[a] | 0.249 ± 0.132 | 6.95 (5.20, 8.70) | 1.40 | 0.157 |
| 4[b] | 0.411 ± 0.220 | 3.97 (2.22, 5.72) | 4.38 | 0.001 |
| 6[b] | 0.697 ± 0.452 | 3.06 (1.31, 4.81) | 5.29 | 0.001 |

LSM, least square mean; CI, confidence interval. For comparative purposes note that $IC_{50}$ COX-2 = 0.394 μg/mL and $IC_{80}$ COX-2 = 1.280 μg/mL in whole blood assays (see Table 3). Treatment groups sharing the same superscript a or b were not significantly different ($P > 0.05$). *Inflammation induced in stifle joint at zero time by intra-articular injection of cytokines induced by lipopolysaccharide in a canine macrophage cell line. †VAS values and mavacoxib concentrations determined 30 days after a second dose of mavacoxib. ‡Difference from placebo.

**Table 5.** Plasma mavacoxib trough concentrations [mean ± SD (*n*)] in osteoarthritis dogs after administration of up to seven doses in Study 1* and five doses in Study 2†

| | | Mavacoxib concentration (μg/mL) | |
|---|---|---|---|
| Dose | Time after dosing (days) | Study 1 | Study 2 |
| 1 | 14 | 1.39 ± 0.40 (210) | 0.52 ± 0.15 (61) |
| 2 | 45 | 1.91 ± 0.74 (203) | 0.73 ± 0.30 (62) |
| 3 | 75 | – | 0.88 ± 0.41 (60) |
| 4 | 105 | 2.45 ± 1.19 (185) | 0.97 ± 0.47 (58) |
| 5 | 135 | – | 1.11 ± 0.50 (56) |
| 6 | 165 | 2.58 ± 1.30 (65) | – |
| 7 | 195 | 2.60 ± 1.58 (98) | – |

*Dosage = 4 mg/kg; †Dosage = 2 mg/kg.

predicted highest maximum plasma concentration would have been 4.16 μg/mL, that is exceeding both the $IC_{20}$ for COX-1 and $IC_{80}$ for COX-2 inhibition. Therefore, it seems very likely that the dose of 4 mg/kg was supramaximal, that is higher than required to achieve effective therapy.

In clinical trials, the percentages of animals improved were 93 and 79 for the 2 and 4 mg/kg dosage, respectively (Payne-Johnson *et al.*, 2009a,b). For the 2 mg/kg dosage group, both owner and veterinary assessments indicated noninferiority to the control dogs receiving carprofen; 93.4% improvement in the mavacoxib group compared with 89.1% in the dogs receiving carprofen for owner assessment. In addition, the improvement in response to mavacoxib increased over the first 6 weeks after commencing therapy and was maintained at a plateau level thereafter. Compared with pretreatment, pain was reduced by 39% after 14 days and by 65% after 135 days from commencement of treatment, when corresponding plasma trough concentrations were 0.52 and 1.11 μg/mL.

*Summary of pharmacological properties of mavacoxib relevant to treatment of canine OA*

A classical dose determination study employing an acute synovitis model in Beagle dogs indicated a mavacoxib dose of 4 mg/kg to be necessary to exhibit pronounced efficacy. Population PKs derived from a field study in which the dose of 4 mg/kg was tested indicated that the plasma elimination half-life was longer in client-owned osteoarthritic dogs than in young healthy Beagle dogs (Cox *et al.*, 2010, 2011). This latter finding made it possible to reduce the dose from 4 to 2 mg/kg and thereby increasing the safety margin while maintaining clinical efficacy. Mavacoxib is licensed for the use for the treatment of pain and inflammation in canine OA, when a treatment period exceeding 1 month is indicated. The dosage schedule is 2 mg/kg on days 1 and 14, and then, the same dosage is administered at 28-day intervals; a treatment cycle should not exceed seven doses. The product literature recommends that clinicians should observe a treatment-free period of at least 1 month before administration of another NSAID after mavacoxib treatment. There are several factors to be considered, when using mavacoxib as an agent for the control of pain and acute inflammatory flare-ups in dogs with OA:

a) Nonsteroidal anti-inflammatory drugs are the drugs of choice for the therapy of canine OA; several have been licensed and all, with the exception of mavacoxib, are recommended for dosing once or twice daily (Sanderson *et al.*, 2009; Innes *et al.*, 2010). Once or twice daily dosing frequency is dictated by the short to intermediate terminal half-lives. Clinically, NSAIDs have been used either to control short-term acute flare-ups in OA or for continuous management over periods of weeks or months. Autefage and Gossellin (2007) reported the maintenance of clinical improvement in OA dogs on long-term carprofen therapy. Similar findings have been reported for mavacoxib (*vide infra*) and may be reflected in improved mobility, reduction in disease muscle atrophy and possibly reduced rate of disease progression (Sanderson *et al.*, 2009; Innes *et al.*, 2010). In terms of therapeutic benefit, there are theoretical efficacy advantages arising from maintained plasma concentrations for mavacoxib, in comparison with the peaks and troughs of concentration provided by daily dosing with other NSAIDs (*vide supra*). However, clinical efficacy can be established only in well-designed comparative clinical trials with effective monitoring of responses and, in this regard, similar efficacy has been demonstrated for carprofen daily and mavacoxib monthly dosing.
b) The inhibition of COX-2, at a level approaching or exceeding 80% throughout a treatment period of up to 6 months, should ensure for mavacoxib an adequate level of pain control throughout. It is theoretically possible that, even with well-maintained plasma concentrations, there could be development of tolerance to the actions of the drug at the molecular level [pharmacodynamic (PD) tolerance]. However, this seems unlikely, based on both theoretical and clinical grounds. Drug–enzyme interactions are rarely associated with tolerance and owner and veterinary assessments of efficacy gave no indication of reduced effect with duration of treatment. The slow rate of decrease in plasma concentration of mavacoxib over the dosing interval should ensure steady maintenance of analgesia.
c) The signs of OA are commonly intermittent rather than continuous. Both laboratory animal studies and clinical experience suggest that continuous analgesic therapy breaks the cycles of acute flare-ups to provide pain control and increased mobility, leading to maintenance of muscle mass, increased joint stability and possible slowing of disease process (Sluka *et al.*, 1994b; Sanderson *et al.*, 2009). The maintenance of an effective plasma concentration of mavacoxib throughout the interdose interval will ensure that tissue (including synovial fluid) concentrations are in equilibrium with those in plasma and thereby minimize variability in concentrations at the site of action.
d) The persistent and prolonged inhibition of prostanoid production by COX-2 enzymes caused by mavacoxib may have therapeutic advantages related to efficacy over intermittent

inhibition. While such persistent inhibition could to a large degree be achieved by constant administration of short-acting NSAIDs, it has to be acknowledged that reduced compliance of daily administration as well as the constantly changing blood concentrations after daily administration may not provide the same level of stable inhibition as long-acting NSAIDs such as mavacoxib. Repeated stimulation of the nervous system with pain stimuli leads to the phenomena of hyperalgesia and allodynia, which occur through adaptive responses in protein expression in peripheral and central neurones, thereby changing how these systems perceive sensory stimuli. Continuous and persistent inhibition of prostanoid production at sites of chronic inflammation, such as joints involving degenerative joint disease, may lead to more effective control of these processes. From the central and peripheral mechanisms contributing to hyperalgesia and allodynia, it is clear that changes in protein expression as a result of inflammatory mediator activation of neuronal pathways underlie complex mechanisms of central and peripheral hyperalgesia. Thus, it is likely that relatively short-term activation of prostanoid receptor pathways could lead to longer lasting hyperalgesic effects through, for example the increase in density of tetrodotoxin-resistant sodium channels or enhanced transmission of pain signals in the spinal cord. The duration of this effect will depend on the rate of neuronal channel or receptor degradation. If clinical compliance on dosing frequency with short-acting NSAIDs is relatively poor, as it will be on occasions, intermittent formation of prostanoids could be sufficient to activate these pathways and perpetuate the phenomenon of hyperalgesia (central or peripheral) possibly even leading to the wind-up phenomenon.

e) The selected clinical dose (2 mg/kg) of mavacoxib inhibits the COX-1 isoform to a much lesser degree than that of COX-2, comprising 20% or less inhibition of the former at trough concentrations. This may minimize side effects on the g.i.t. and blood clotting mechanisms attributable to inhibition of this isoform. However, it is possible that hypercoagulable states might be worsened. Moreover, it should be noted that COX-2 is constitutive in several tissues and, as with other selective COX-2 inhibitors, the persistent action of mavacoxib might inhibit certain physiological or pathophysiological functions under certain conditions. The possible disadvantage of mavacoxib, potentially, is its irreversibility, should side effects occur in clinical use in an individual patient; treatment will have to be symptomatic. Nevertheless, clinical experience with mavacoxib to date would suggest that the prevalence, duration and outcome of suspected adverse reactions, occurring in dogs receiving mavacoxib, is no different to those occurring in dogs treated with carprofen long term on a daily dosage regimen (Six *et al.*, 2012).

f) The side effect of NSAIDs of greatest incidence and severity, comprising damage to the upper g.i.t. arising from their irritant actions, may be minimized by the long dosage intervals (14 days after dose 1 and 28 days thereafter) of orally administered mavacoxib. Damage may arise from three major causes: (i) exposure of the mucosal lining of the stomach and intestine to high local concentrations after dosing and prior to absorption, (ii) exposure via local blood flow containing the drug and (iii) local mucosal exposure through daily drug secretion in bile (Whittle, 2004). With mavacoxib, the local exposure associated with dosing is intermittent and therefore reduced in comparison with the once or twice daily dosage regimens required for other NSAIDs licensed to treat canine OA. However, exposure to mavacoxib through the blood supply is continuous rather than phasic. Moreover, exposure to semicontinuous low concentrations is likely as a consequence of biliary excretion. On the other hand, for those drugs with daily dosing recommendations but with short terminal half-lives, there is a potential advantage of daily periods of low exposure of the g.i.t. from all three sources.

g) The low frequency of dosing with mavacoxib should ensure good owner compliance with the recommended dosing schedule. As discussed by Lees and Maddison (2006), there is evidence of poor compliance with dosing schedules in canine medicine, especially when oral administration is more than once daily. In fact, Payne-Johnson *et al.* (2009a, b) demonstrated that the dosage regimen of 2 weeks between first and second and 4 weeks between subsequent doses did provide greater compliance than once daily NSAID dosing. Whereas noncompliance with number of and/or interval between doses might raise welfare/efficacy issues for short-acting NSAIDs, a delay of 2–3 days in administering the next dose of mavacoxib should not have significant implications for efficacy.

## CONCLUSION

Mavacoxib is a novel NSAID, with a preferential action on the COX-2 isoform of COX and a long duration of action. The dosage schedule of mavacoxib for clinical use has been determined by owner and veterinary clinical assessments and is supported by integration of PK and PD preclinical data with clinical responses in canine disease models and in dogs with naturally occurring OA. The dosage regimen has been further confirmed by correlating levels of inhibition of COX isoforms in *in vitro* whole blood assays with plasma concentrations of mavacoxib achieved in OA dogs. Integration of PK and PD data suggests that the recommended dosage regimen of 2 mg/kg bw once for 14 days, followed by administration at monthly intervals, is optimal for both efficacy and safety perspectives and is further confirmed by clinical field studies.

## DECLARATIONS OF INTEREST

J. Elliott and P. Lees have acted as consultants and have conducted research funded by Pfizer Animal Health. L. Pelligand has conducted research funded by Pfizer Animal Health. M.

Stegemann and G. Michels are employees of Zoetis, formerly known as Pfizer Animal Health.

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