Assessment of the Efficacy of Firocoxib and Robenacoxib in an Induced Synovitis Model of Acute Arthritis in Dogs

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ABSTRACT

The objective of this study was to compare the analgesic activity of a single oral dose of two COX-2 selective inhibitors, firocoxib (Previcox®, Merial) and robenacoxib (Onsior®, Elanco), in an acute pain model in dogs. Sixteen healthy Beagle dogs were randomly allocated to three groups. Two successive experiments were conducted, in which eight dogs served as control. Eight dogs received firocoxib or robenacoxib in a cross-over design at the recommended dosage 13 hours before intra-articular injection of a urate crystal suspension (UC) for induction of synovitis. Ground reaction forces (Peak Vertical Force, PVF) and clinical Visual Lameness Scores (VLS) were measured before induction of synovitis, at 1.5, 3, 5, 7, 10, and 24 hours after UC injection (except PVF which was not measured after 10 hours). In this study, pretreatment with firocoxib significantly reduced the acute pain and lameness induced by UC injection, as shown by the decreased combined visual score of lameness at 3 hours post-injection, and the increased PVF values compared to the control group at 3 and 5 hours post-injection. Firocoxib performed significantly better than robenacoxib at 3, 5, and 10 hours post-UC injection. In this model, robenacoxib was not different from control for both the VLS and the PVF values. Pre-treatment with firocoxib reduced the induced pain associated with intra-articular administration of urate crystals.

INTRODUCTION

Chronic osteoarthritis is common in dogs and is estimated to affect 20% of dogs over 1 year of age (Johnston, 1997). Since no drug has been shown to reverse the pathological changes of osteoarthritis, the objective of treatment is to reduce pain and inflammation, and thus maintain the dog's mobility and quality of life (Kukanich et al., 2012).

Non-steroidal anti-inflammatory drugs (NSAIDs) are the most widely used analgesics in veterinary medicine. They are commonly used in the treatment of acute pain following surgical and dental procedures and are the cornerstone in the treatment of osteoarthritis and other painful conditions. The main mechanism of action of NSAIDs is in

the inhibition of cyclooxygenase (COX), an enzyme in the arachidonic acid cascade, which generates inflammatory mediators of the prostaglandin (PG) group (Vane, 1971; Lees et al., 2004).

Two isoforms of COX have been identified. Cyclooxygenase-1 (COX-1) is present constitutively in almost all cell types (excluding erythrocytes) and is mainly involved in maintaining physiologic functions, including gastroprotection and maintenance of renal homeostasis, and is implicated in blood clotting (Jones et al., 2000; Lees et al., 2004). Cyclooxygenase-2 (COX-2) is induced in inflammatory responses producing pro-inflammatory PGs, such as PGE2 (Jones et al., 2000; Lees et al., 2004). It is generally accepted that inhibition of COX-1 is likely to account for most of the side-effects of NSAIDs (gastric irritation, renal damage, and prolonged bleeding time) and that their efficacy is mainly dependent on the inhibition of COX-2 (Warner et al., 1999; Lees et al., 2004).

Intensive efforts have been made to develop specific inhibitors of the COX-2 isoform while sparing the activity of COX-1. Firocoxib (Previcox®, Merial) and robenacoxib (Onsior®, Elanco) are highly selective inhibitors of COX-2 developed specifically for veterinary use (McCann et al., 2004; King et al., 2009). Both products are registered for once daily administration (24 hourly). In whole blood canine assays (the gold standard for assessing COX activity), the COX-2 selectivity, determined on the basis of the COX-1: COX-2 ratio of the IC50 values (inhibition of 50% of COX activity) was 384 for firocoxib (McCann et al., 2004) and 128.8 for robenacoxib (King et al., 2010). In vivo, firocoxib and robenacoxib induce marked inhibition of COX-2, while sparing COX-1, when administered at clinically recommended dosages (McCann et al., 2004; King et al., 2010; Schmid et al., 2010; King et al., 2011). The efficacy and safety of firocoxib and robenacoxib in the dog have been extensively demonstrated in experimental clinical studies (McCann et al, 2004; Drag et al, 2007; Steagall et al, 2007; Hazewinkel et al, 2008; Schmid et al, 2010; King et al, 2011) and in field studies (Hanson et al, 2006; Pollmeier et al, 2006; Ryan et al, 2006; Joubert, 2009; Ryan et al, 2010; Autefage et al, 2011; Reymond et al; 2012; and Edamura et al, 2012).

The specific objective of the present study was to evaluate the daily analgesic activity (potency and persistence of analgesia over 24 hours) of a single oral dose of firocoxib and robenacoxib in an acute synovitis model. To assess the ability of each NSAID to control pain over 24 hours, each was administered 13 hours prior to the induction of acute inflammation by the intra-articular injection of urate crystals

MATERIALS AND METHODS

Animals

Animal housing and care complied with the recommendations of Directive 86/609/EEC. The animal care and use program was AAALAC accredited. The study plan was approved by the AmatsiAvogadro Animal Ethic Committee.

Sixteen male Beagle dogs, aged from 16 to 28 months and weighing from 8.5 to 11 kg, were included. The animals were selected based on a full clinical examination and blood (haematology and blood chemistry) analyses. Particular attention was paid to the locomotory system of the enrolled animals to ensure the absence of lameness before inclusion. Animals were housed individually throughout the study and fed with a commercially available dog food with unlimited access to water. Animals were identified using a microchip.

During the acclimatization period (3 weeks) before the first experiment, dogs were trained to walk on a lead during the first week and to walk on the force plate in the two following weeks.

Study design and treatments

The study was divided into two experiments (Exp1 and Exp2) separated by a 2 month wash out period in order to reduce the number of dogs included in the study for ethical

Table 1. *Allocation of the 16 dogs in the different groups.*

Experiments	Untreated control dogs	Firocoxib treated dogs	Robenacoxib treated dogs
Exp.1	1, 2, 3, 4	5, 6, 7, 8	13, 14, 15, 16
Washout period (2 months)			
Exp.2	9, 10, 11, 12	13, 14, 15, 16	5, 6, 7, 8

reasons. Each experiment included three groups of four dogs: four untreated control dogs, four treated with firocoxib, and four with robenacoxib. During the second experiment, four new control dogs were included, whereas the four dogs treated with firocoxib corresponded to the four previously treated with robenacoxib, and vice versa (Table 1). The washout period and cross over design allowed the inclusion of fewer dogs but also to consider that the variances would be similar between the treated groups.

Sixteen dogs were ranked by weight and then randomized into three groups of four dogs in Exp 1 and Exp 2. For analysis, the data from the two experiments were pooled. Treatments were administered orally 13 hours before UC injection in each experiment. Animals were fasted 7 hours before firocoxib or robenacoxib administration, and overnight before each UC injection. The dogs were fed approximately 1 hour after each UC injection on day 1. Firocoxib and robenacoxib were administered at the approved label dosages for the control of pain and inflammation associated with osteoarthritis. Firocoxib (Previcox®, Merial) was given at a dose of 1 tablet of 57 mg for dogs with a bodyweight ranging from 5.6 to 10.0 kg and 1 + 1/2 tablet of 57 mg for dogs with a bodyweight ranging from 10.1 to 15.0 kg (targeted therapeutic dose of 5mg/kg, with a range of 5-10 mg/kg, ie, ranging 6.4-8.3 mg/kg in the present study). Robenacoxib (Onsior®, Elanco) was administered at a dose of one tablet of 10 mg for dogs with a bodyweight ranging from 5.0 to 10.0 kg and one tablet of 20 mg for dogs with a bodyweight ranging from 10.1 to 20 kg (targeted therapeutic dose 1mg/kg with a range of 1 to 2mg/kg, ie, ranging 1-1.9 mg/kg in the present study).

Experimental Model

Lameness was induced 13 hours after test drug administration using an established reversible urate crystal (UC) arthritis model (Toutain et al, 2001).

The sodium UC suspension (Sigma Aldrich, USA) was prepared to a final concentration of 10 mg/mL according to the published method (Toutain et al, 2001). One mL of suspension was used for each intra-articular injection. Dogs were anesthetized with propofol intravenously at a dose of 6.5 mg/kg, before the intra-articular injection of UC.

All UC injections were administered into the right stifle (femoro-tibial) joint under aseptic conditions using 19-gauge sterile needles (30 mm long) and 2.5-mL syringes.

Using this model, the duration of the induced inflammatory and painful process is approximately 16-24 hours, with a pain intensity peak within 2 to 3 hours after induction in untreated animals (Toutain et al., 2001).

Efficacy Assessment

The anti-inflammatory and analgesic efficacy of the test items was assessed through two parameters: the Visual Lameness Score (VLS) and the Peak Vertical Force (PVF). The VLS was assessed for each period on the day before induction (Day 0 before UC injection), and 1.5h, 3h, 5h, 7h, 10h, and 24h post-UC injection (corresponding to 14.5h, 16h, 18h, 20h, 23h, and 37h after test drug administration). The PVF was assessed for each period, on the day before induction (Day 0 before UC injection), and 1.5h, 3h, 5h, 7h, and 10h post-UC injection (corresponding to 14.5h, 16h, 18h, 20h, and 23h

Table 2. *Description of Visual Lameness Scoring.*

Scoring parameters	**Score**
Observation whilst standing	**0 to 2**
Full weight bearing (touching of the 4 digital pads on the ground)	0
Partial weight bearing (touching of 3 or fewer digital pads on the ground)	1
No weight bearing to toe touching	2
Observation whilst walking	**0 to 3**
Full weight bearing, no lameness	0
Slight lameness (including intermittent) with partial weight bearing (75%): lameness barely perceptible throughout almost the whole observation period	1
Moderate lameness with partial weight bearing (≥ 50%): the animal rests the limb on the ground slightly	2
Severe lameness with no weight bearing: the animal uses its limb but it does not put its weight on the limb and/or avoids putting the limb on the ground	3
Combined Lameness score	**0 to 5**

after drug administration).

The VLS was assessed by four trained investigators (same person for dog training and trial) using a scoring system during standing and walking. The combined Visual Lameness Score was the sum of the standing and walking phase scores (ranging from 0 to 5) (Table 2).

The PVF (expressed in Newton) was the force applied to the ground plate for the induced hind limb during walking. The measurement section was composed of eight force plates with a size of (0.2 x 0.2) m² each, located on teo walkways (0.8 x 0.2 m in total). The force plate section was inserted in a path of 50 cm wide and of 5 m long to allow the dog to walk with a constant and homogenous speed when it arrived on the measurement section located in the middle of the path on which the dogs were trained to walk. The SATEL (SATEL-Patrick Savet, Blagnac, France) force plate was connected to a computer equipped with a digital analogical acquisition card and a signal processing software (Satel Véto, Ecole Vétérinaire de Toulouse, France). Dogs were filmed during walking to retrospectively validate the recorded values.

Dogs had to walk five times down the path of the force plate at each time point to obtain at least three interpretable values for each hind limb. Validation of values was based on the walking video and on the curve appearance generated by the software.

The peak vertical force ratios correspond to the peak force applied at different time-points after UC intra-articular injection and the peak force of the same hind limb in the absence of inflammation (Toutain et al, 2001). The PVF ratios were calculated for different time-points for the treated and the untreated dogs. The PVF ratios of severe lameness with no weight bearing observed during walking phase (no PVF recorded) was considered equal to zero.The investigators making the efficacy assessments were blinded to treatment groups.

Analysis of the Results

Statistical analyses were carried out using R Statistical Software. The level of significance was set at $p \leq 0.05$. Considering that clinical responses to pain in dogs should be normally distributed, and that the crossover design allows to hypothesize similar

Table 3. *Mean combined Visual Lameness Scores observed during the study.*

Time-points	Control dogs Mean / SD	Firocoxib treated dogs Mean / SD	Robenacoxib treated dogs Mean / SD
0h	0 / 0	0 / 0	0 / 0
1.5h	1.25 / 1.16	0.63 / 1.77	1.5 / 2.2
3h	**3.88[a] / 1.36**	**0.75[a] / 1.49**	**3.5[a] / 2.27**
5h	2.5 / 1.85	0.875 / 1.13	2.63 / 2.33
7h	1.63 / 1.85	0.5 / 0.76	2.38 / 2.56
10h	1 / 1.85	0.25 / 0.71	1.75 / 2.19
24h	0 / 0	0 / 0	0 / 0

[a] Significant difference between the 3 groups (ANOVA, p = 0.0033), between firocoxib treated dogs and controls (Student, p = 0.00061), and between the two treatment groups (Student, p = 0.012).

variances between the two treated groups, ANOVA test was used to compare the three groups of eight dogs, and the Student t Test was performed to compare each treatment group from the controls, as well as the treatment groups together.

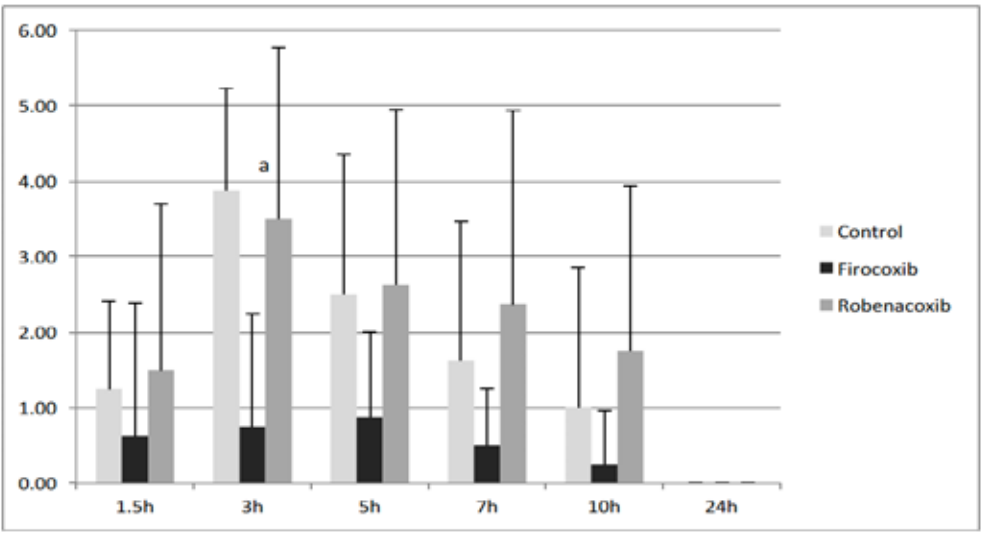
RESULTS

Visual Lameness Scores

On day 0, before UC injection, all animals were free of lameness (ie, baseline VLS = 0).

Observed collectively, the three groups significantly differed at 3h time-point after induction of synovitis (p = 0.0033) (Table 3, Figure 1). There was no significant difference between robenacoxib treated dogs and untreated control dogs at any time-points. Firocoxib treated dogs showed a significantly better combined lameness score at 3h after UC injection (p = 0.00061). The two treatment groups were also significantly different at the 3h time point (i.e, 16h post-treatment) (p = 0.012).

Figure 1. *Mean combined Visual Lameness Scores per group observed during the study.*



a Significant difference between the 3 groups (ANOVA, p = 0.0033), between firocoxib treated dogs and controls (Student, p = 0.00061), and between the two treatment groups (Student, p = 0.012).

Table 4. *Mean Peak Vertical Force ratio (%) observed during the study.*

Time-points	Control dogs Mean / SD	Firocoxib treated dogs Mean / SD	Robenacoxib treated dogs Mean / SD
1.5h	82.6 / 13.7	78.7 / 33.1	60.7 / 38.8
3h	**38.0 / 34.7**[a]	**79.6 / 23.1**[a]	**29.1 / 41.2**[a]
5h	**56.6 / 36.7**[b]	**88.6 / 13.8**[b]	**50.4 / 46.0**[b]
7h	71.3 / 32.4	90.7 / 15.3	53.8 / 46.9
10h	80.8 / 24.1	**93.9 / 13.4**[c]	**55.9 / 47.8**[c]

[a] *Significant difference between the 3 groups (ANOVA, p = 0.016), between firocoxib treated dogs and controls (Student t test, p = 0.014), and between the two treatment groups (Student, p = 0.009).*
[b] *Significant difference between firocoxib treated dogs and controls (Student, p = 0.037), and between the two treatment groups (Student t test, p = 0.041).*
[c] *Significant difference between the two treatment groups (Student t test, p = 0.048).*

Peak Vertical Force Ratios

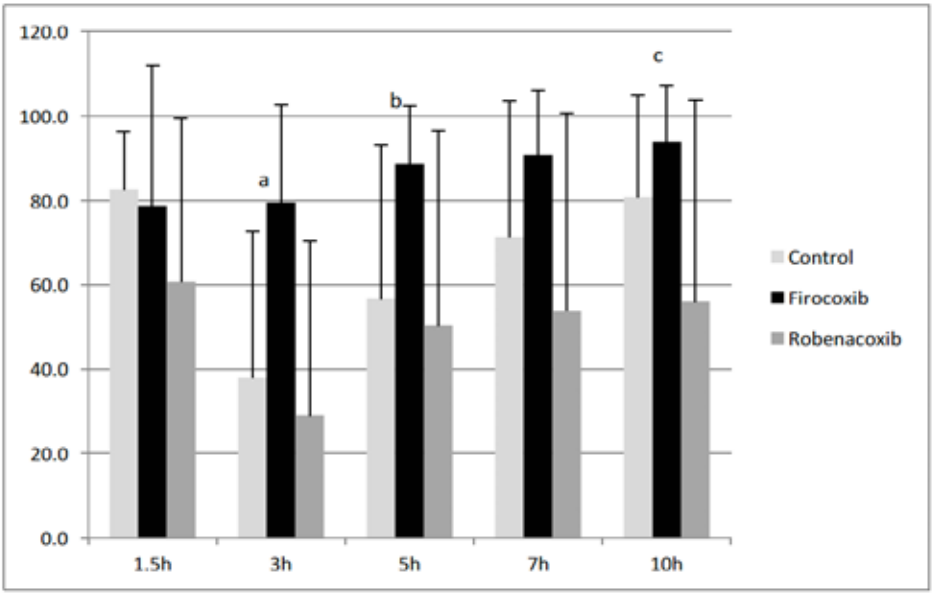
On day 0, before induction of joint pain, baseline PVF were not significantly different between groups.

Observed collectively, the PVF ratios of the three groups were significantly different at 3h time-point after UC injection (16h post treatment) (p = 0.016) (Table 4, Figure 2).

There was no significant difference between robenacoxib treated dogs and untreated dogs at any time-points concerning the PVF ratios.

Firocoxib treated dogs showed a significantly better PVF ratio than untreated dogs at 3h and 5h after UC injection (p = 0.014, and p = 0.037, respectively). The two treat-

Figure 2. *Mean Peak Vertical Force ratio (%) observed in the 3 groups of 8 dogs during the study.*



[a] *Significant difference between the 3 groups (ANOVA, p = 0.016), between firocoxib treated dogs and controls (Student t test, p = 0.014), and between the two treatment groups (Student, p = 0.009).*
[b] *Significant difference between firocoxib treated dogs and controls (Student, p = 0.037), and between the two treatment groups (Student t test, p = 0.041).*
[c] *Significant difference between the two treatment groups (Student t test, p = 0.048).*

ment groups were significantly different at 3h, 5h, and 10h post UC injections (ie, 16h, 18h, and 23h post treatment) ($p = 0.009$, $p = 0.041$, and $p = 0.048$, respectively).

DISCUSSION

The intra-articular injection of urate crystals induced consistent lameness and pain in all controls, which peaked approximately 3h after injection, as observed in other studies (Toutain et al, 2001). This 3h period post UC injection and 16h post treatment correspond to the time-point where significant differences were observed between the three groups in both the combined lameness scores and the peak vertical force ratios.

All dogs gradually recovered within 24 hours after UC injection, which was clearly observed in the combined lameness scores that were back to 0 at thee aformentioned period. It can be noted that the dose used in our study was 10 mg, as described by Toutain et al (2001), which results in a decrease in severity and length of lameness compared to the standard dose of 19 mg used in others studies (McCann et al, 2004; Drag et al, 2007; Hazewinkel et al, 2008).

The lameness scoring system, by nature, remains subjective and is based on two parameters only, walking and standing dogs. It can explain the high variability observed between dogs, as demonstrated by the standard deviations, and therefore the difficulty to obtain significant differences.

In contrast, the Peak Vertical Force system is an objective measure. It can thus be expected that variability would be lessened and standard deviations lower than with a subjective scoring system. As expected, in this study, the analysis of PVF allowed better comparison of the three groups. Firocoxib treatment performed significantly better than robenacoxib treatment at the 3h, 5h, and 10h time points (ie, 16h, 18h, and 23 hours after drug administration), and significantly better than the negative control at 3h and 5h time points (ie, 16 and 18 hours after product administration).

In this study, the administration of firocoxib significantly reduced the acute pain and lameness induced by the UC injection compared to control dogs at 3h and 5h time-points (i ,. 16h and 18 hours after product administration). It confirmed the efficacy of firocoxib in the management of acute pain previously demonstrated in a UC-induced synovitis model (McCann et al, 2004; Drag et al, 2007; Hazewinkel et al, 2008) and under field conditions (Hanson et al, 2006; Pollmeier et al, 2006; Ryan et al, 2006; Joubert, 2009; Ryan et al, 2010; Autefage et al, 2011). More importantly, it confirmed sustained pain control several hours after administration and the rationale for daily administration.

The statistical difference between the firocoxib and robenacoxib PVF ratio at 10h post UC injection (i.e, 23 hours after treatment administrations) showed that firocoxib was still providing efficacious analgesia 23h post administration. In the dog, the half-life is shorter for robenacoxib (0.8h) (Jung et al, 2009) than firocoxib (5.9h) after oral administration (McCann et al, 2004), which may explain the observed differences in this study. The present study focused only on acute pain control after a single administration. The efficacy of firocoxib and other COX-2 selective inhibitors should also be assessed in future studies using this UC model after several days of repeated administrations.

CONFLICT OF INTEREST STATEMENT

This clinical study was funded by Merial S.A.S., 2 avenue Pasteur, 69007 Lyon of which Christelle Dauteloup and Frederic Beugnet are employees.

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All authors voluntarily published this article and have no personal interest in these studies other than publishing the scientific findings that they have been involved in via planning, initiating, monitoring and conducting the investigations and analysing the results.

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