Pharmacokinetics and ex vivo pharmacodynamics of oral firocoxib administration in New Zealand White rabbits (*Oryctolagus cuniculus*)

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OBJECTIVE
To examine the pharmacokinetics and ex vivo pharmacodynamics of oral firocoxib administration in New Zealand White rabbits (*Oryctolagus cuniculus*).

ANIMALS
6 healthy New Zealand White rabbits.

PROCEDURES
Pharmacokinetics were determined from plasma concentrations measured via ultra performance liquid chromatography-tandem mass spectrometry after oral administration of firocoxib at a dose of 3.74 to 4.20 mg/kg. Pharmacokinetic analysis was performed using non compartmental methods. Pharmacodynamics of firocoxib were evaluated by measuring plasma concentrations of thromboxane and prostaglandin via ELISAs as surrogate markers of cyclooxygenase enzyme isoform inhibition.

RESULTS
The terminal rate constant was 0.07 hours (range, 0.05 to 0.11 h). The mean maximum concentration (Cₘₐₓ) and time to Cₘₐₓ were 0.16 µg/mL and 3.81 hours (range, 2.0 to 8.0 h), respectively. Mean residence time was 15.02 hours. Mean elimination half-life was 9.12 hours. For the pharmacodynamic analysis, firocoxib administration did not demonstrate a significant difference between any time point for prostaglandin E₂ and only a significant difference between 24 and 48 hours for thromboxane B₂.

CLINICAL RELEVANCE
Although the pharmacokinetic research supports that plasma firocoxib concentrations that would be therapeutic in dogs are achieved in rabbits, the pharmacodynamic results do not demonstrate a significant difference in levels of cyclooxygenase-2 inhibition, which indirectly reflects the anti-inflammatory effects of the drug. Further pharmacodynamic studies and multidose studies are warranted to determine the efficacy and safety of this drug in rabbits.

Nonsteroidal anti-inflammatory drugs (NSAIDs) are important therapeutic agents that offer significant benefits in rabbit medicine for control of inflammation, pain, and fever.¹² The principle action of NSAIDs is inhibition of the cyclooxygenase (COX) enzymes. The COX enzymes are important in catalyzing a key step in converting arachidonate to prostaglandin (PG) H₂, which acts as an immediate substrate in the biosynthesis of prostaglandins and thromboxane.³⁴ Prostaglandins are crucial to a variety of biologic processes, which include regulating immune function, renal development, reproductive biology, and the integrity of the gastrointestinal tract.¹

There are three isoforms of COX enzymes: COX-1, COX-2, and COX-3. COX-1 is constitutively expressed in a large number of tissues, and its activity is responsible for the production of cytoprotective prostaglandins that are critical to the maintenance of the gastric mucosa, maintenance of renal blood flow and glomerular filtration rate, and production of the precursors for synthesis of thromboxane, a potent inducer of platelet aggregation.⁴ COX-2 is an inducible enzyme that plays a major role in inflammation and neoplasia and becomes upregulated during times of tissue damage, which results in the generation of proinflammatory PGs, including PGE₂ and PGF₂α.⁵ Many of the well-known and undesirable adverse effects of NSAIDs may be attributed to inhibition of the constitutive COX-1 isoform enzyme and include renal toxicity, platelet inhibition, and
gastrointestinal ulceration with subsequent perforation; however, inhibition of the COX-2 isoform has also been reported to produce undesirable effects.\textsuperscript{6–8} NSAIDs have been demonstrated to be either nonselective, COX-2 preferential, or COX-2 selective.\textsuperscript{10,11} The COX-2 selective inhibitors have been demonstrated to have fewer side effects reported compared to those seen with nonselective COX-inhibitors, making them more desirable and clinically useful to inhibit inflammation at the site of tissue damage.\textsuperscript{6} Caution is required with the use of any NSAID to monitor for any adverse effects that may occur, regardless of its selectivity.\textsuperscript{2}

Firocoxib is an NSAID that selectively inhibits the COX-2 isoform, with sparing of COX-1 isoform inhibition.\textsuperscript{15} In dogs, firocoxib has been demonstrated to be 384 times more selective for the canine COX-2 isoform than for the COX-1 isoform.\textsuperscript{10,11} Firocoxib has US FDA approval in the United States for control of pain and inflammation associated with osteoarthritis in dogs and horses. Firocoxib is available as an oral paste for horses, a tablet for dogs, and as an injection for both species. The oral dose of firocoxib selected for this study was based on a comparison of equine and canine dosing, tablet size, and the suspected pharmacokinetics and pharmacodynamics.\textsuperscript{3,7,9,14–18}

The objective of the study reported here was to evaluate the pharmacokinetics of a single oral dose of firocoxib to New Zealand White rabbits. Additionally, the study aimed to investigate the magnitude and time course of inhibition of ex vivo plasma TXB\textsubscript{2} formation and plasma PGE\textsubscript{2} formation, as indicators of COX-1 and COX-2 inhibition, respectively. The oral dose of firocoxib selected for this study was determined based on a comparison of equine and canine dosing, tablet size, and the suspected faster metabolism known in smaller species.\textsuperscript{14,16,21,22} It was hypothesized that firocoxib administered orally in rabbits at a dose of 3 to 4 mg/kg would maintain therapeutic plasma concentrations for 12 to 24 hours when interpreted in conjunction with the pharmacodynamic results. Additionally, it was hypothesized that TXB\textsubscript{2} concentrations would be minimally affected by firocoxib and PGE\textsubscript{2} concentrations would significantly decrease following firocoxib administration.

Materials and Methods

Six clinically normal, specific-pathogen-free (\textit{Pasteurella} sp), 9-month-old New Zealand White rabbits (\textit{Oryctolagus cuniculus}), consisting of 3 males and 3 females and ranging in weight from 3.39 to 3.81 kg, were obtained from a commercial supplier and included in this study. The rabbits were transferred from a previous study protocol (noninvasive, pharmacokinetic study), and therefore, a period of 1 month was allowed to elapse prior to the start of the study. Each rabbit was housed individually in a temperature- and humidity-controlled room consisting of a stainless-steel cage for 12 hours per day and an indoor run measuring 7.8 X 0.9 X 1.8 m for the other 12 hours of the day. The photoperiod for each rabbit was 12 hours of light and 12 hours of dark on an automatic timer setting. The diet and husbandry were standardized for each of the 6 rabbits. Each rabbit was fed a commercial pelleted diet and ad libitum timothy hay. None of the rabbits were fasted prior to initiation of the study, and food and water were provided throughout the duration of the study.

One week prior to the start of the study, a physical examination and assessments of PCV and total protein were performed to verify that each rabbit was healthy. During blood collection for PCV and total protein, blood was also obtained (total 1 mL) for blank plasma samples. The study was approved by the Kansas State University Institutional Animal Care and Use Committee (protocol No. 4527). Rabbits were adopted after completion of the study (1 to 2 mo later).

Dosage selection, drug administration, and blood sample collection

Each rabbit was manually restrained and administered one-quarter of a 57-mg tablet (dose range, 3.74 to 4.20 mg/kg; median, 3.87 mg/kg) of firocoxib (Previcox; Merial) with the use of a cat pill gun, followed by 3 mL of water via syringe to ensure swallowing. Each rabbit was then restrained for 2 minutes following administration to ensure that all rabbits swallowed the medication completely. A blood sample (1 mL) was collected with a needle and syringe from the lateral saphenous or cephalic vein immediately before (0 minutes) and at 15 minutes, 30 minutes, and 1, 1.5, 2, 4, 6, 8, 12, 24, and 48 hours after firocoxib administration for pharmacokinetic analysis and an additional 0.5 mL was collected at 2, 4, 8, 12, 24, and 48 hours for pharmacodynamic analysis. Collected blood samples were placed into lithium heparin tubes without separators and stored on ice until further sample processing.

Monitoring

All rabbits were monitored during the time periods prior to, during, and after the study for signs of adverse effects, including changes in mentation, eating or drinking behavior, or urination or defecation.

Pharmacokinetic analysis sample processing

The plasma was separated within 1 hour of collection via centrifugation for 12 minutes at 2,600 rpm. The plasma was harvested and stored in cryogenic vials at −80°C for 2 weeks until time of sample analysis for pharmacokinetics and ex vivo pharmacodynamics.
Plasma firocoxib determination

Firocoxib analysis was conducted using previously described methods developed in swine. Briefly, frozen samples and standards (firocoxib and firocoxib-d3; Sigma-Aldrich) were thawed at room temperature and rigorously vortexed once completely thawed. The plasma, calibration, blank (negative rabbit plasma), and quality control samples were extracted and cleaned up by solid phase extraction using an Oasis HLB Prime µElution 96-well plate (Waters Co). Subsequently, 100 µL of each firocoxib working solution, the quality control solution, and the rabbit plasma sample was added into a 48-well nontissue culture-treated plate, respectively. Fifty microliters of the internal standard solution was loaded into each well. To adjust the total volume to 300 µL, 150 µL of 4% phosphoric acid was added into each well, and then the 48-well plate was shaken gently at 350 rpm for 20 min at room temperature with the IKA MTA 2/4 Shaker. The 48-well plate was then centrifuged at 2,000 x g for 30 min at 20°C. The supernatant was transferred to the wells of the Oasis HLB Prime µElution 96-well plate. Following a wash with 300 µL of 5% methanol, both the target components and internal standards were eluted with 50 µL 90/10 acetonitrile/methanol. Then, 50 µL of 2mM ammonium formate containing 0.1% formic acid (pH 4.0) was added into each well of the 96-well square collection plate and shaken gently at 300 rpm for 3 minutes at room temperature with the IKA MTA 2/4 Shaker (IKA Works Inc) before the ultraperformance liquid chromatography-electrospray ionization-tandem mass spectrometry (UPLC-MS-MS) analysis.

The UPLC-MS-MS analysis was carried out with a Waters Acquity Ultra Performance LC (with a Waters column manager and heater/cooler, binary system manager, and sample manager coupled to a Waters Xevo TQ-S triple quadrupole mass spectrometer equipped with electrospray ionization (ESI; Xevo TQ-S MS/MS/Waters Co). The C18 column (Acquity UPLC HSS T3 column, 1.8 µm, 2.1 x 50 mm) was held at 40°C with eluents composed of mobile phase A (2 mM ammonium formate combined with 0.1% formic acid and pH adjusted to 4.0 using ammonium hydroxide and mobile phase B (acetonitrile). The flow rate was 0.4 mL/min. Following gradient program was used: 0 from 0 to 0.8 minutes phase A (70%), at 0.8 minutes phase A (30%), hold for 1.2 minutes, and at 2.01 minutes phase A (70% for 0.99 min). Total run time was 3 minutes. The triple quadrupole mass spectrometer was Xevo TQ-S MS/MS equipped with an ESI interface. This ionization mode used was positive ionization (⁺ESI). The operating parameters for the mass spectrometer were as follows: capillary voltage, 3.60 kV; source and desolvation temperatures, 150°C and 600°C, respectively. The cone energy was set at 50 V for firocoxib and firocoxib-d3. Nitrogen was used as the desolvation gas and cone gas. Helium was used as the collision gas. The collision gas flow was 0.15 mL/min. Desolvation and cone gas flow were 1,000 and 150 L/h, respectively. Ions were monitored in the multiple reaction monitoring mode with transitions at m/z 337.10 → 129.81 (qualifier), 337.10 → 282.96 (quantifier) for firocoxib; and m/z 343.16 → 135.74 (qualifier), 343.16 → 289.03 (quantifier) for firocoxib-d6. The data acquisition and quantification were performed using the Waters MassLynx and TargetLynx software 4.1, respectively (Waters Co).

The calibration curve was linear from 0.1 to 1,000 ng/mL with the correlation coefficient (R²) exceeding 0.99. The lower limit of quantitation for firocoxib was 0.1 ng/mL, and the lower limit of detection was 0.05 ng/mL. The interday precision was 1.87%, 4.55%, 5.82%, and 3.46% at 1, 10, 100, and 500 ng/mL, respectively. The accuracy was 93.87%, 96.65%, 101.73%, and 103.81% at 1, 10, 100, and 500 ng/mL, respectively.

Firocoxib pharmacokinetic analysis

Pharmacokinetic analysis for each rabbit was performed using computer software (PK Solver). For each rabbit, the plasma firocoxib concentration time curve was used for noncompartmental analysis with uniform weighting based on statistical moment theory. The maximum concentration (Cmax) and time to Cmax (tmax) were determined by visual inspection of the data. The terminal half-life was determined by log linear regression using the last 3 to 5 time points of firocoxib concentrations. Individual animal pharmacokinetic values were determined, and descriptive statistics were reported.

Determination of plasma PGE₂ and TXB₂ concentrations

Plasma PGE₂ concentrations were determined using methods previously described. In brief, prior to sample processing, lipopolysaccharide (LPS) solution from *Escherichia coli* O111:B4 was created to a concentration of 1 mg/mL by adding 10 mL of phosphate-buffered saline with a serological pipette using 10 mg of LPS. Subsequently, 1 mL of fresh whole blood was aliquoted from the heparinized green top tube using a micropipette set to 1 mL and placed in an Eppendorf tube. Ten microliters of 1 mg/mL LPS was pipetted with a micropipette and added to the Eppendorf tube. The tube was mixed thoroughly by at least 3 to 5 inversions. If any samples were short, the appropriate ratio of LPS to sample was utilized. The tubes were then placed on a raft in a warm water bath at 37°C for 24 hours. After 24 hours, each sample was removed from the warm water bath and processed via centrifugation for 12 minutes at 2,600 rpm. The plasma was harvested and stored in cryogenic vials at −80°C until time of sample analysis.

Plasma PGE₂ and TXB₂ concentrations were determined using commercially ELISA kits (Cayman Chemical). Both ELSIA kits use anti-mouse IgG harvested from goats that have been validated for rabbits. For PGE₂ determination, 375 µL of plasma was mixed with 1.5 mL of ethanol (dilution, 1:5) to precipitate proteins. Similarly, for TXB₂ determination, 375 µL of plasma was mixed with 1.5 mL acetonitrile (dilution, 1:5) to precipitate proteins. The solution was vortexed at a low speed to mix thoroughly. No inverting

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of tubes was done to avoid sample leakage. The tubes were centrifuged at 2,500 X g for 10 minutes, and the supernatant was collected. Then, 100 or 500 µL was aliquoted into glass tubes (13 X 100 mm) and evaporated down using a nitrogen dryer. The manufacturer’s ELISA protocol was then followed. The volume utilized (100 to 500 µL) depended on whether the PGE$_2$ or TXB$_2$ concentrations exceeded the upper limit of detection and required dilution to fit the standard curve. If a sample was above the detection range, then it was reanalyzed at a lower volume. The dilution factor was recorded to allow the actual concentration to be calculated. Absorbance was measured at 420 nm after development. The intra-assay and interassay coefficients of variation for PGE$_2$ were 10.4% and 2.7%, respectively. The intra-assay and interassay coefficients of variation for TXB$_2$ were 11.7% and 10.3%.

**Statistical analyses**
Plasma PGE$_2$ and TXB$_2$ concentrations were calculated as percent change from baseline as follows: (sample PGE$_2$ or TXB$_2$ − baseline PGE$_2$ or TXB$_2$)/baseline PGE$_2$ or TXB$_2$ X 100. Repeated-measures ANOVA with Greenhouse-Geisser correction and Tukey multiple comparisons post hoc analysis were performed to determine if percent change in PGE$_2$ and TXB$_2$ differed between 0, 2, 4, 8, 12, 24, and 48 hours. Statistical significance was set at $P \leq 0.05$ a priori. Repeated measures ANOVA was performed using GraphPad Prism 9.2 (GraphPad Software).

**Results**
Throughout the duration of the experiment (1 mo prior to the start of the study and 1 to 2 mo following completion of the study), all rabbits appeared to remain healthy (no evidence of diarrhea, anorexia, or lethargy) with no obvious adverse effects of drug administration.

**Pharmacokinetics**
A semilogarithmic plot of plasma firocoxib concentrations over time after oral firocoxib administration (dose range, 3.74 to 4.20 mg/kg) for rabbits was created (Figure 1). The pharmacokinetic parameters of firocoxib following oral administration were presented as geometric means (Table 1). The individual animal data were tabulated (Supplementary Table S1). The mean C$_{\text{max}}$ and t$_{\text{max}}$ were 0.16 µg/mL (range, 0.14 to 0.17 µg/mL) and 3.81 hours (range, 2 to 8 h), respectively. Mean residence time was 15.02 hours. Mean elimination half-life was 9.12 h (range, 6.48 to 14.08 h). Firocoxib was detected in all 6 rabbits at 48 hours with a mean ± SD concentration of 0.0071 ± 0.0047 µg/mL (range, 0.0016 to 0.0155 µg/mL).

**Pharmacodynamics**
To determine the magnitude of COX inhibition by firocoxib, individual animal data for each of the 6 rabbits were analyzed. The mean time course following firocoxib administration of 7 time points was selected for subsequent pharmacodynamic analysis. These time points demonstrated the mean percent change in the values of TXB$_2$ and PGE$_2$ from baseline over a 48-hour period. The maximum reduction in PGE$_2$ concentrations was noted at the 12-hour time point and was less than 50% of baseline (Figure 2). Thromboxane concentrations were

![Figure 1: Mean ± SD plasma firocoxib concentration at various points following oral administration of a single dose of firocoxib (3.74 to 4.20 mg/kg) to 6 New Zealand White rabbits (Oryctolagus cuniculus). Time of firocoxib administration was designated as time 0.](image)

**Table 1—Summary data for pharmacokinetic parameters for firocoxib following oral administration of a single dose of firocoxib (3.74 to 4.20 mg/kg) to 3 male and 3 female New Zealand White rabbits (Oryctolagus cuniculus).**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean*</th>
<th>Minimum</th>
<th>Median</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC$_{\text{extrap}}$ (%)</td>
<td>2.82</td>
<td>0.70</td>
<td>3.83</td>
<td>10.29</td>
</tr>
<tr>
<td>AUC$_{\text{extrap}}$ (µg·h/mL)</td>
<td>2.57</td>
<td>2.14</td>
<td>2.58</td>
<td>3.08</td>
</tr>
<tr>
<td>AUMC (µg·h$^2$/mL)</td>
<td>38.59</td>
<td>23.94</td>
<td>40.40</td>
<td>65.88</td>
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<tr>
<td>C$_{\text{max}}$ (µg/mL)</td>
<td>0.16</td>
<td>0.14</td>
<td>0.17</td>
<td>0.17</td>
</tr>
<tr>
<td>Half-life (h)</td>
<td>9.12</td>
<td>6.48</td>
<td>10.19</td>
<td>14.08</td>
</tr>
<tr>
<td>λ$_z$ (1/h)</td>
<td>0.05</td>
<td>0.07</td>
<td>0.11</td>
<td></td>
</tr>
<tr>
<td>MRT (h)</td>
<td>15.02</td>
<td>11.17</td>
<td>15.05</td>
<td>21.49</td>
</tr>
<tr>
<td>t$_{\text{max}}$</td>
<td>3.81</td>
<td>2.0</td>
<td>4.0</td>
<td>8.0</td>
</tr>
</tbody>
</table>

λ$_z$ = Terminal rate constant. AUC$_{\text{extrap}}$ = Percentage of the area under the concentration-versus-time curve extrapolated to infinity. AUC$_{\text{extrap}}$ = Area under the concentration-versus-time curve from time 0 to infinity. AUMC = Area under the first moment curve extrapolated to infinity. C$_{\text{max}}$ = Maximum concentration; MRT = Mean residence time. t$_{\text{max}}$, time to C$_{\text{max}}$.

*All means are presented as geometric mean except half-life, which is presented as harmonic mean.
maximally reduced at 48 hours at approximately 25% of baseline. No significant difference between any time point and the percent reduction for PGE₂ concentration was noted whereas only a significant (P = 0.04) difference between 24- and 48-hour time points for TXB₂ concentration was detected.

**Discussion**

To the authors’ knowledge, this is the first report examining the pharmacokinetics and the ex vivo pharmacodynamic effect of firocoxib, a selective COX-2 inhibitor, in domestic rabbits. The inhibition of COX-1 and COX-2 was indirectly examined by evaluation of plasma concentrations of TXB₂ and PGE₂, respectively. NSAIDs are one of the most commonly administered classes of analgesic and anti-inflammatory drugs in rabbits, yet data regarding newer, selective NSAID usage in rabbits are limited.28–35 There are few studies that evaluate the pharmacokinetic and pharmacodynamic interactions of NSAIDs in rabbits. The findings from the present study aimed to address some of the challenges associated with rabbit analgesia encountered on a daily basis. In general, it is worth noting that rabbits appear to tolerate NSAIDs well with minimal side effects reported in this species.28–31 There is a single report36 of gastric ulceration and perforation in a rabbit that received chronic meloxicam. The vast majority of drugs that are used in rabbit medicine are used off-label; however, procedures are routinely performed in rabbits that induce pain and inflammation and warrant analgesic and anti-inflammatory medications.35

In the present study, firocoxib absorption reached a maximum concentration at 3.81 hours (t_{max}) following oral administration. When the pharmacokinetic results of firocoxib in the study rabbits were compared with data from horses and dogs, there were similarities and differences noted. The t_{max} varied across species reflecting a difference in rate of absorption in dogs (t_{max} mean, 1.25 h), horses (t_{max} mean, 3.2 ± 1.09 h), and rabbits (t_{max} mean, 3.81 h).14,15,36 The differences in t_{max} and the wide range in the t_{max} for the rabbits in this study are likely due to the sampling time points and the wide variation that is common with an observed parameter such as t_{max}. Additionally, individual variation may also account for some of the differences that were seen. The C_{max} in rabbits (mean, 0.16 µg/mL) was higher than that seen in horses (mean, 0.058 ± 0.032 µg/mL), but lower than dogs (mean, 0.52 ± 0.22 µg/mL), which may be a reflection of the dose used in this study being closer to the dose that is commonly used in dogs (5 mg/kg), compared to horses (0.1 mg/kg).14,15

The mean plasma elimination half-life in the study rabbits (mean ± SEM, 9.12 ± 1 h), which describes the time that is required for plasma drug concentrations to be reduced by half, was higher than in dogs (mean ± SEM, 5.9 ± 1.1 h)15 and camels (mean ± SEM, 5.7 ± 2.2 h),27 but much shorter compared to horses (mean ± SEM, 32.77 ± 10.74 h),14 swine (mean ± SD, 24.075 ± 9.47 h),23 and calves (mean, 18.8 h).38 It is desirable to have a longer terminal half-life because this may translate to a longer duration of analgesia and anti-inflammatory effects following administration of a single dose, resulting in less frequent dosing requirements. The shorter half-life in rabbits compared to many of the other species that have been previously studied may indicate that rabbits require either larger maintenance doses or more frequent drug administration.

The elimination half-life of drugs and their metabolites usually occurs due to biotransformation of the drug, followed by renal and/or biliary excretion.39 There are known species differences in biliary excretion of drugs, with rabbits being poor biliary excretors and dogs being good biliary excretors as a result of several adenosine triphosphate-binding cassette transmembrane transporters that have differing activity and regulation among species.39 Some species, such as dogs, appear to have a much greater level of biliary excretion of the drug compared to horses, which could account for differences seen in the elimination half-life between species.15 Additionally, it is known that elimination of firocoxib is primarily in the urine, with some level of biliary excretion, which may account for some of the differences seen among species.15 It is also well known that rabbits often require different dosages of drugs compared to other species, often higher than canine and feline patients.39–42 Differences in dosage result from a combination of factors including animal size on metabolic rate, with larger animals requiring lower doses of drugs; protein binding; physicochemical properties of the drug; and differences in drug transport and metabolism.39

The exposure of oral firocoxib reflected in the area under the curve in rabbits (2.57 µg·h/mL) is less than that reported in dogs (4.75 ± 0.42 µg·h/mL) but more than that reported in horses (0.787 ± 0.227 µg·h/mL).10,14 Indirectly, this could indicate that rabbits clear the drug from the body faster than dogs but slower than horses; however, clearance...
was not directly measured in the present study. This information could be used in future studies to help infer the dosing interval of the drug in rabbits.

The findings in the present study demonstrated that there was a greater inhibition of the COX-2 enzyme compared to the COX-1 enzyme (Figure 2). It is not possible to state precisely the level and duration of COX-2 inhibition that should be achieved for provision of suitable levels of inhibition of inflammation, fever, or appropriate analgesia. Additionally, although the effect on COX-1 inhibition appears to be less, it is not known what the threshold of inhibition is to minimize or eliminate the risk of adverse effects on the gastrointestinal tract or impairment of hemostatic mechanisms. In other studies, the recommendation is that COX-1 inhibition time is minimized and that it should not exceed 10% to 20% at the peak concentration of the drug. In the study presented here, during times of peak drug concentrations (2 to 12 h), TXB2 was inhibited at 2 hours and 12 hours with no inhibition occurring at 4 and 8 hours after drug administration.

In dogs, COX-2 inhibition assays have reported both effective in vivo and therapeutic plasma concentrations (for experimentally induced synovitis) in a range from 30 to 67 ng/mL. This level was achieved between 0.5 and 2.0 hours after oral administration in the rabbits in this study and was maintained until 24 hours in all but 1 rabbit. When these data are examined in conjunction with the pharmacodynamic data, dosing intervals for the drug can be inferred. Although some inhibition of PGE2 is seen for approximately 12 hours, the difference between any time points is not significant, suggesting the current dosing regimen may not be sufficient for therapeutic efficacy for analgesia. Multidose studies have been performed in horses, where an accumulation index of 4.00 was determined for the oral tablet formulation, demonstrating that the drug does demonstrate bioaccumulation. Future studies in rabbits should include multidose and safety studies to better determine the time interval for frequency of administration of the drug and if bioaccumulation also occurs in the rabbit. Given the presence of plasma concentrations that are present at 48 hours (Figure 2), there is consideration for bioaccumulation of this drug in the rabbit.

Results of studies performed in both humans and dogs have demonstrated the benefits of selective COX-2 inhibitors in terms of adverse effects from a gastrointestinal perspective. Although neither endoscopy or necropsy was performed to verify an absence of gastrointestinal tract lesions, no obvious changes were noted in dietary intake, and the rabbits maintained normal fecal output throughout the duration of the study.

Limitations of this study reported here included a small sample size. The smallest possible number of rabbits was used to complete the pharmacokinetic and pharmacodynamic analysis, but a larger sample size would have been advantageous to better evaluate interindividual variability. The absence of a pilot study for dose selection, as well as the absence of a control group, was also a limitation of this study. Additionally, biochemistry profiles were not performed on the rabbits throughout the study to evaluate for renal and hepatic toxicity. Although exact measurements of food intake and fecal output were not undertaken during the duration of the study, each rabbit was monitored closely for any changes in amount eaten or decreases in fecal output; however, without specific monitoring, all possible adverse events were not fully evaluated for. Further research in rabbits should be performed to evaluate safety of this drug, as well as pharmacokinetics of firocoxib after administration of multiple doses that may demonstrate bioaccumulation.

Although the pharmacokinetic data from this study supported that therapeutic levels for analgesia are achieved in rabbits when compared to other species, the pharmacodynamic results did not demonstrate a significant difference in levels of COX-2 inhibition, which indirectly reflects the anti-inflammatory effects of the drug. Future studies should focus on clinical pharmacodynamic anti-inflammatory and analgesic effects of the drug to correlate these clinical considerations with the concentration of the drug in plasma.

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**References**


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Supplementary Materials

Supplementary materials are posted online at the journal website: avmajournals.avma.org

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