Plasma pharmacokinetics and synovial fluid concentrations after oral administration of single and multiple doses of celecoxib in Greyhounds

Robert P. Hunter, PhD; MaryAnn Radlinsky, DVM, MS; David E. Koch, MS; Matthew Corse, DVM; Marie A. Pellerin, BA; Jennifer Halstead, BS

Objective—To determine the plasma pharmacokinetics and synovial fluid concentrations after oral administration of single and multiple doses of celecoxib in Greyhounds.

Animals—7 adult Greyhounds.

Procedures—Dogs received celecoxib (median dose, 11.8 mg/kg [range, 11.5 to 13.6 mg/kg], PO, q 24 h) for 10 days. Blood samples were collected prior to administration of celecoxib and serially for 24 hours after the 1st and 10th doses were administered. A synovial joint catheter was placed into a stifle joint in each dog for collection of synovial fluid samples. Concentrations of celecoxib in plasma and synovial fluid were quantified by use of a validated liquid chromatography/mass spectrometry method. Identification of hydroxy- and carboxyl-celecoxib in plasma and synovial fluid was also performed. Pharmacokinetic parameters were determined by use of noncompartmental analysis.

Results—Administration of multiple doses of celecoxib resulted in a significant decrease (40%) in median maximum concentration (Cmax; 2,620 to 2,032 ng/mL) between the 1st and 10th doses. Blood concentrations after the 1st and 10th doses were administered. A synovial joint catheter was placed into a stifle joint in each dog for collection of synovial fluid samples. Concentrations of celecoxib in plasma and synovial fluid were quantified by use of a validated liquid chromatography/mass spectrometry method. Identification of hydroxy- and carboxyl-celecoxib in plasma and synovial fluid was also performed. Pharmacokinetic parameters were determined by use of noncompartmental analysis.

Conclusion and Clinical Relevance—Celecoxib distributes into the synovial fluid of Greyhounds. Although the exact mechanism for the decreases in AUC and Cmax is not known, results suggested that the plasma pharmacokinetics of celecoxib are different after administration of multiple doses in Greyhounds. These findings warrant further investigation on the absorption, distribution, metabolism, and elimination of celecoxib in Greyhounds and other breeds of dogs. (Am J Vet Res 2005;66:1441–1445).

Reportedly, pharmacokinetic parameters and drug metabolism differ considerably among breeds of dogs. Greyhounds metabolize cyclizine (a histamine receptor 1 antagonist) differently than other breeds of dogs or species. Barbiturates have also been reported to have different pharmacokinetics in Greyhounds, compared with other breeds of dogs. The plasma clearance of pentobarbital and methohexital is slower in Greyhounds than mixed-breed dogs with a corresponding increase in mean residence time. Propofol hydroxylase activity is 3-fold lower in Greyhounds than in Beagles. Because of selective breeding over several centuries, it is possible that differences in renal or hepatic physiology have developed in Greyhounds, leading to differences in xenobiotic distribution, metabolism, and excretion, compared with other breeds of dogs.

Presently, Greyhounds are treated with various nonsteroidal anti-inflammatory drugs (NSAIDs), which are not specific cyclooxygenase inhibitors, despite the fact that these drugs can have serious adverse effects, even when used at the labeled dose. Celecoxib may be an alternative treatment for inflammation in racing greyhounds. Celecoxib is metabolized in humans by cytochrome P-450 2C9 and in Beagles by cytochrome P-450 2D15 to hydroxy-celecoxib and carboxyl-celecoxib, then subsequent noncytochrome P-450 mediated phase II glucuronide conjugation of carboxylic-celecoxib occurs. Metabolites are excreted in urine and feces; however, excretion in feces is the predominant route. Two distinct phenotypes have been detected in laboratory-raised Beagles and humans, those that metabolize celecoxib extensively and those that metabolize celecoxib poorly.
Information on the pharmacokinetics of celecoxib in plasma and the concentrations of celecoxib in synovial fluid may be beneficial in guiding the use of celecoxib in racing Greyhounds and providing additional information regarding pharmacokinetic variability between dog breeds. The purpose of the study reported here was to determine the plasma pharmacokinetics and the synovial fluid concentrations after oral administration of single and multiple doses of celecoxib in Greyhounds. To the authors’ knowledge, this is the first study to determine the concentrations of celecoxib in synovial fluid in any species. The information generated should permit rational dose design and may assist in establishing associations for extrapolation and use of other therapeutic agents in Greyhounds.

Materials and Methods

Dogs—Seven adult Greyhounds, 2 females and 5 males, weighing from 25 to 37 kg were used in the study. Dogs were determined to be healthy on the basis of results of clinical examination and CBC and serum biochemical analysis. The study was approved by the Institutional Animal Care and Use Committee of Kansas State University.

Surgery for placement of synovial joint catheters—During general anesthesia, a synovial joint catheter was placed into 1 stifle joint of each dog by use of a modification of the method described by Wigness et al. A polytetrafluoroethylene (PTFE)-lined, silicone rubber tube (0.36 × 12.7 mm) with a threaded rigid plastic segment was implanted in the femoral condyle with its outlet port in the stifle joint. Briefly, during general anesthesia, a standard lateral approach to the stifle joint was performed and a small hole was drilled through the lateral femoral condyle in a lateral to medial direction. The catheter tip was screwed into the condyle until the outlet port entered the joint without contacting the condylar or tibial joint surfaces. The cranial and caudal cruciate ligaments and the medial and lateral menisci were protected with hemostatic forceps placed medial to the medial aspect of the lateral femoral condyle. The inlet catheter was tunneled subcutaneously to a conveniently located site in the subcutaneous tissue over the lateral femoral condyle. The length of the catheter was shortened and attached to an implanted access port. The access port was sutured to the deep fascia to maintain the port position, and the subcutaneous tissue and skin were closed taking care to avoid the access port site.

After surgery, each dog received buprenorphine (0.01 mg/kg, SC, q 12 h for 24 hours). All dogs were monitored hourly for the first 3 hours and then every 4 hours for the next 24 hours. Dogs were then monitored daily for the next 2 weeks for signs of infection and inflammation. The ports were flushed weekly with heparin (400 U/mL) to maintain patency. An interval of at least 4 weeks was permitted from the time of surgery until the start of the study. Cytologic examination of synovial fluid was performed every 3 days (<0.5 mL) for the first 2 weeks after surgery, and results were within reference limits by the end of this period. The volume of the catheter and port was determined in each dog and accounted for during sample collection.

Sample collection and pharmacokinetics of celecoxib in plasma—Celecoxib (100- and 200-mg capsules, PO) was administered to each dog at a median dose of 11.8 mg/kg (range, 11.5 to 13.6 mg/kg) with a dosing interval (τ) of 24 hours for 10 days. Food was not withheld for the study, and celecoxib was administered 1 hour prior to feeding. Blood samples (5 mL) were collected via venipuncture into lithium heparin evacuated tubes. After administration of the 1st and 10th doses of celecoxib, blood samples were collected prior to administration of the next dose of celecoxib and at 0.25, 0.5, 1, 2, 4, 6, 8, 12, and 24 hours after administration and prior to and 1 hour after administration of the third, fifth, and seventh doses of celecoxib. Synovial fluid samples (<0.5 mL) were collected prior to administration of celecoxib; 1 and 12 hours after the first dose was administered; 1 hour after the third, fifth, and seventh doses were administered; and at 1, 12, 24, and 48 hours after the 10th dose of celecoxib was administered. Plasma was separated via centrifugation (approx 2,000 × g for 10 minutes) within 1 hour of blood collection. Plasma and synovial fluid samples were stored at –70°C until analyzed.

Pharmacokinetic parameters were determined for each dog by use of noncompartmental analysis2,22 with a commercial software program. Parameters calculated for plasma included area under the plasma concentration versus time curve (AUC), and area under the moment curve (AUMC). Mean residence time (MRT) was calculated by use of the equation MRT = AUMC/AUC. Maximum concentration (Cmax) of celecoxib in plasma and the time (Tmax) occurred were read directly from the data. The terminal rate constant (λ) was determined from the slope of the terminal phase of the plasma concentration curve that included a minimum of 4 points. The apparent half-life (t1/2) was calculated by use of t1/2 = 0.693/λ after the 1st and 10th doses and is reported as a harmonic mean. The AUC and AUMC after the 1st and 10th doses were calculated by use of trapezoidal rule with and without extrapolation to infinity to permit appropriate comparison between the 1st and 10th doses.2,22

Celecoxib analysis—Concentrations of celecoxib in plasma and synovial fluid were quantitated by use of the method of Paulson et al; modified and validated in plasma and synovial fluid from Greyhounds for liquid chromatography/mass spectrometry (LC/MS) analysis. The LC/MS system used a PBD (50 × 2.1 mm; 3 μm) column. The mobile phase consisted of a 40:60 ratio of acetonitrile and 10mM ammonium acetate and a flow rate of 0.3 mL/min. The MS ion-trap detector was set as follows: single ion monitoring for celecoxib and metabolites; m/z (mass-to-charge ratio) at 308.2 (nimesulide, internal standard), 380.2 (celecoxib), 396.2 (hydroxy-celecoxib), 410.2 (carboxyl-celecoxib), and 586.2 (glucuronide conjugate of carboxyl-celecoxib); collision energy, 35%; ionization source, electrospray ionization; source voltage, 4.0 kV; sheath gas flow rate, 95 arbitrary units; auxiliary gas flow rate, 33 arbitrary units; capillary voltage, 25 V; and capillary temperature, 235°C.

Standards and quality control (QC) samples were prepared by use of acetonitrile to dissolve a known weight of celecoxib analytical standard in a 5-mL volumetric flask. This primary stock solution was used for making plasma standards and QC samples. Plasma from Greyhounds was used for all standard curves. The assay was linear across the range of the standard curve of 5 to 3,000 ng/mL, with a limit of quantitation of 5 ng/mL. Intra- and interday accuracy and precision were less than ±10% and 8% of intended values, respectively. Recovery of celecoxib from plasma was >72% across the range of these QC assays. The QC samples were assayed in duplicate and were interspersed throughout the analytical run.

The samples, standards, and QC samples extracted were 500-μL aliquots placed in centrifuge tubes. Fifty microliters of internal standard (1 μg of nimesulide/mL) was added to each tube. Five hundred microliters of 0.1M acetic acid (pH, 5.0) was then added, and the plasma solution was mixed again. To this, 200 μL of 2-propanol and 3 mL of toluene were added. The mixture was vortexed for 2 minutes, then centrifuged for 10 minutes at 2,000 × g. The supernatant was transferred to clean tubes and dried under N2 at 50°C water bath. The samples were reconstituted in...
100 µL of mobile phase, vortexed for 1 minute, and transferred to autosampler vials, and 50 µL was injected onto the LC/MS system. All samples from a single dog were analyzed by use of a single run. Celecoxib is reportedly stable in plasma for at least 13 weeks at –70°C,23,24 and all samples were assayed within 2 months of collection. The described method was also used to identify the hydroxy and carboxylic acid metabolites of celecoxib on the basis of published daughter spectra and m/z.25,26

Statistical analyses—Pharmacokinetic values obtained after administration of the 1st and 10th doses of celecoxib were compared by use of the nonparametric Wilcoxon matched-pairs signed rank test.27 Values of P < 0.05 were considered significant. Values for the various pharmacokinetic parameters are reported as mean ± SD, median, and range.

Results

Signs of pain, abnormal gait, or inflammation were not detected in any dog during the study. Mean ± SD concentrations of celecoxib in plasma and synovial fluid were determined (Figures 1 and 2). The concentration of celecoxib in synovial fluid was 145 ng/mL 1 hour after the first administration of celecoxib. The concentration of celecoxib (515 ng/mL) in synovial fluid peaked 1 hour after the fifth dose of celecoxib was administered and decreased to 306 ng/mL 1 hour after the 10th dose of celecoxib was administered.

Administration of multiple doses of celecoxib resulted in a significant decrease in median AUC and AUMC values (P = 0.008) and a corresponding decrease in median Cmax values (P = 0.023) between the 1st and 10th doses, independent of the time (0 to infinity or t) over which the AUC was determined (Table 1). None of the other pharmacokinetic parameters (apparent t½, λ, T max, and MRT) were significantly different between administration of the first and last doses of celecoxib. Concentrations of celecoxib in synovial fluid were less than the corresponding plasma concentrations at all times except at 24 hours after the last dose was administered.

Both hydroxy- and carboxy-celecoxib metabolites were consistently detected in plasma and synovial fluid. Quantitation was not possible because of the lack of analytical standards. The analytical method used in this study did not yield any samples in which the carboxylic acid glucuronide conjugate was detected.

Table 1—Plasma pharmacokinetic parameters after administration of the 1st and 10th doses of celecoxib (median dose, 11.8 mg/kg [range, 11.5 to 13.6 mg/kg], PO, q 24 h for 10 days) in Greyhounds (n = 7).

<table>
<thead>
<tr>
<th>Pharmacokinetic parameters</th>
<th>1st dose</th>
<th>10th dose</th>
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</thead>
<tbody>
<tr>
<td>Mean ± SD</td>
<td>Median</td>
<td>Range</td>
</tr>
<tr>
<td>AUC∞ (h X ng/mL)</td>
<td>33,213 ± 23,831</td>
<td>29,112*</td>
</tr>
<tr>
<td>AUMC∞ (h² X ng/mL)</td>
<td>252,307 ± 238,771</td>
<td>216,355*</td>
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<tr>
<td>AUCt (h X ng/mL)</td>
<td>32,045 ± 21,889</td>
<td>28,878*</td>
</tr>
<tr>
<td>AUMCt (h² X ng/mL)</td>
<td>214,861 ± 166,656</td>
<td>209,686*</td>
</tr>
<tr>
<td>λ (h⁻¹)</td>
<td>0.175 ± 0.0359</td>
<td>0.175</td>
</tr>
<tr>
<td>t½ (h)</td>
<td>4.0T</td>
<td>4.0</td>
</tr>
<tr>
<td>T max (h)</td>
<td>2.6 ± 0.96</td>
<td>2.0</td>
</tr>
<tr>
<td>Cmax (ng/mL)</td>
<td>3,907 ± 2,227</td>
<td>2,620*</td>
</tr>
<tr>
<td>MRT (h)</td>
<td>7.1 ± 1.2</td>
<td>6.8</td>
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*Significantly (P < 0.05) different from median value after 10th dose. Harmonic mean.

AUC∞ = Area under the curve from 0 to infinity for the first dose and from 216 hours to infinity for the 10th dose. AUMC∞ = Area under the moment curve from 0 to infinity for the first dose and from 216 hours to infinity for the 10th dose. AUCt = Area under the curve from 0 to 24 hours for the first dose and from 216 to 240 hours for the 10th dose. AUMCt = Area under the moment curve from 0 to 24 hours for the first dose and from 216 to 240 hours for the 10th dose. λ = Elimination rate constant. t½ = Half-life. Cmax = Maximum concentration in plasma. T max = Time Cmax occurred. MRT = Mean residence time (AUMC∞/AUC∞).
Discussion

The study reported here yielded pharmacokinetic, synovial distribution, and limited celecoxib metabolism data after oral administration of single and multiple doses of celecoxib to adult Greyhounds. Celecoxib rapidly distributed into the synovial fluid after oral administration. Concentrations of celecoxib in plasma decreased after 10 days of treatment as a result of a mechanism that is yet unknown.

The $t_{1/2}$ of 4 hours determined in the study reported here is similar to that reported for Beagles (5.1 hours) that poorly metabolize celecoxib. The $C_{\text{max}}$ (2,620 ng/mL) after the first dose was 8 to 9 times greater in Greyhounds, compared with the $C_{\text{max}}$ in Beagles (280 to 320 ng/mL) receiving celecoxib at an actual dose of 12.5 mg/kg every 12 hours. Results of another study in Beagles that metabolize celecoxib both poorly and extensively indicate that AUC and $C_{\text{max}}$ increased after the first dose and remained increased until after the 360th dose of celecoxib (12.5 mg/kg, PO, q 12 h) was administered, which is different from results of our study.

Multiple factors may explain the differences in $C_{\text{max}}$ and AUC between the 1st and 10th doses of celecoxib in Greyhounds. The AUC is a parameter used to represent systemic exposure, and AUC depends on the dose, rate of absorption, and rate of elimination from the sampled compartment (plasma). The $\lambda$, apparent $t_{1/2}$, and $T_{\text{max}}$ were not significantly different between the 1st and 10th doses. These data lead to several hypotheses that may explain the differences in $C_{\text{max}}$ and AUC. Intestinal absorption may have decreased between doses 1 and 10. The possible decrease in bioavailability (AUC) may have resulted from an inhibitory effect on absorption of celecoxib from the small intestine, such as competitive inhibition of a recirculating metabolite on the transport of celecoxib from the intestinal lumen into the blood; however, to the authors’ knowledge, this has not been previously reported for any pharmaceutical.

Bioavailability after oral administration depends on the fraction absorbed from the gastrointestinal tract and the fraction escaping first-pass metabolism. It is possible that dissolution of celecoxib in the stomach or small intestine changed between the 1st and 10th doses. Capsules approved by the FDA for use in humans were used in the study reported here. All dogs were clinically normal during the study as determined on the basis of results of daily observations. Although possible, it is unlikely that a change in gastrointestinal dissolution of celecoxib in all 7 dogs is the explanation for the decrease in AUC and $C_{\text{max}}$ in our study.

Another explanation for the decrease in AUC and $C_{\text{max}}$ is that first-pass metabolism was altered by celecoxib in Greyhounds after repeated dosing, resulting in a decrease in AUC and $C_{\text{max}}$ from the 1st to the 10th dose. With cytochrome P-450 induction, a larger fraction of absorbed celecoxib would have been metabolized and eliminated, never reaching the systemic circulation, resulting in a decrease in $C_{\text{max}}$ and AUC.

Dogs were treated identically and fed at the same time and in the same manner throughout the study. Feed intake did not change during the study. To the authors' knowledge, celecoxib has not been reported to induce any cytochrome P-450 isoforms, although it has been reported to inhibit cytochrome P-450 2D6 in humans. The problem with this theory is that the $\lambda$ and apparent $t_{1/2}$ did not change, which would typically be expected if clearance of celecoxib changed.

The change in the pharmacokinetics of celecoxib after repeated dosing in Greyhounds appears to add evidence against the use of extrapolation from 1 species or breed to another. Beagles, similar to humans, have 2 distinct phenotypes, those that metabolize celecoxib poorly and those that metabolize it extensively. Differences in cytochrome P-450 2D15 expression or the ability of celecoxib to induce cytochrome P-450 2D15 in Greyhounds may explain the results of our study. To the authors’ knowledge, the induction of cytochrome P-450 2D15 by any xenobiotic has not been reported.

Although uncommon, another NSAID, E5110, reportedly induces its own metabolism in Beagles. Results of that study indicate that both $C_{\text{max}}$ and AUC decreased by at least 30% after 15 days of administration of E5110 at all dosages (1 to 250 mg/kg, PO, q 24 h for 13 weeks). There was also a significant difference in the extent of induction of E5110 between sexes. This induction increased from day 15 to 91. Similar to results of our study, no change in $T_{\text{max}}$ as a result of the induction was detected. However, the $t_{1/2}$ and $\lambda$ were not reported, making it difficult to compare results of that study with results of our study.

Because cyclooxygenase-2 appears to be associated with joint inflammation as a result of acute or chronic insult, potential therapeutic agents must reach this site to be effective. In the past, measurement of drug concentrations in synovial fluid has been performed in samples obtained via repeated arthrocentesis, which has historically been performed during general anesthesia and may lead to inflammation and infection of the joint.

Sample volumes collected by use of arthrocentesis are typically ≥ 50 µL, which is similar to volumes collected via the synovial port system used in this study. In the study reported here, placement of the synovial port system decreased many of the risks associated with repeated arthrocentesis. This method should assist with the development of humane and less labor-intensive synovial fluid collection for research in dogs. The synovial port system should also facilitate the development of synovial fluid pharmacokinetic studies to model the distribution of NSAIDs in normal or inflamed joints.

The movement of molecules between the synovial fluid and plasma is determined by molecular size, lipophilicity, and pKa (pH at which the molecule is 50% ionized) with small, polar molecules being cleared by venular reabsorption and proteins by lymphatic drainage. Inflammatory disease may increase the total concentration of celecoxib in synovial fluid and decrease the unbound fraction. We believe that the use of this chronic synovial port system may facilitate basic and clinical research to determine appropriate markers of inflammatory joint disease, drug distribution into the synovial space, and development of inflammatory models of joint disease that will benefit dogs and humans.

The synovial port system described in the study
reported here has been used to infused therapeutic agents into the synovial space. Because of the small volume of fluid in stifle joints of Greyhounds, we modified the procedure of Wigness et al to permit synovial fluid collection. These alterations permitted repeated sampling of synovial fluid and did not appear to cause overt inflammation or trauma to the stifle joint. Because celecoxib is highly protein bound in humans, low concentrations of celecoxib in synovial fluid are not unexpected and are likely to be a function of simple diffusion of the plasma-free fraction into the synovial space. In the study reported here, celecoxib concentrations in synovial fluid were less than the corresponding plasma concentrations, similar to results for other NSAIDs in humans.

To the authors' knowledge, this is the first study to describe the plasma pharmacokinetics and synovial fluid concentrations after oral administration of single and multiple doses of celecoxib in Greyhounds. The Cmax and AUC for celecoxib in plasma decreased after repeated administration in Greyhounds. To our knowledge, this has not been reported for celecoxib in any other species or breed of dog. Results of our study also indicated that celecoxib distributed into the synovial fluid of Greyhounds. Additional research is required to investigate cytochrome P-450 isoform identification and regulation in various dog breeds. This study, in addition to other known physiologic and anatomic differences between dog breeds, suggests the possibility that dog breeds may be more accurately described as subspecies of Canis familiaris and that additional research is required to investigate this theory.

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5. Zirchrom PBD column, Zirchrom Separations Inc, Anoka, Minn.

6. WinNonlin, version 4.0.1, Pharsight, Mountain View, Calif.

7. OrthoCath, Esox Technology Corp, Eagan, Minn.

8. Le Port CompanionPort, Norfolk Vet Products, Skokie, Ill.


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13. WinNonlin version 4.0.1, Pharsight, Mountain View, Calif.

14. WinNonlin version 4.0.1, Pharsight, Mountain View, Calif.

15. Zirchrom PBD column, Zirchrom Separations Inc, Anoka, Minn.

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19. Zirchrom PBD column, Zirchrom Separations Inc, Anoka, Minn.


