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Pharmacokinetics of robenacoxib after a single intramuscular dose in smooth dogfish (*Mustelus canis*)

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OBJECTIVE
To determine the pharmacokinetics of robenacoxib after a single intramuscular dose (4.0 mg/kg) in smooth dogfish (*Mustelus canis*).

ANIMALS
8 healthy adult male smooth dogfish in human care within the same habitat.

METHODS
All sharks received a single intramuscular dose of robenacoxib (4.0 mg/kg) in the right caudolateral epaxial musculature. Blood samples were collected under manual restraint from the ventral tail vessel at 30 minutes, 1 hour, 2 hours, 4 hours, 8 hours, and 24 hours after drug administration. Plasma drug concentrations were determined by HPLC followed by noncompartmental pharmacokinetic analysis of the data.

RESULTS
A maximum plasma concentration of 1.24 µg/mL was reached at a mean time of 30 minutes following robenacoxib administration with a plasma elimination half-life of 3.79 hours. Plasma concentrations did not fall below the lower limit of quantification (0.1 µg/mL) at the time points sampled in this study.

CLINICAL RELEVANCE
Intramuscular administration of a single dose (4.0 mg/kg) of robenacoxib in smooth dogfish resulted in rapid absorption to a maximum concentration at approximately 30 minutes after administration and persisted above levels considered to be therapeutic in domestic species for at least 8 hours.

Keywords: smooth dogfish, *Mustelus canis*, elasmobranch, robenacoxib, shark

Elasmobranchs, including shark and ray species, are commonly housed in zoos and aquariums, and in a review of diseases identified in elasmobranchs in human care, infectious and inflammatory states were the most prevalent conditions diagnosed.[1,2] Traumatic injuries were described as the third most prevalent condition, with injuries occurring from both exhibit design and conspecifics.[1,3] Therefore, there is a great need for pharmacokinetic studies to guide the proper use of analgesic and anti-inflammatory therapeutics in elasmobranchs in order to improve the health and welfare of these species.[4] While the interest and focus on fish analgesia is increasing, there is still limited research on this topic.[4,5]

NSAIDs are a common option for both analgesia and the reduction of inflammation in humans and animals. Broadly, NSAIDs work through the inhibition of the cyclooxygenase (COX) enzyme. This enzyme exists in 2 forms, COX-1 and COX-2, and is responsible for the production of prostaglandins within the body. The COX-1 isoform is constitutively expressed in the vasculature of the kidney, within platelets, and is involved in the production and regulation of gastric acid.[6,7] The COX-2 isoform is considered to be induced in many tissues, functioning in states of inflammation, pyrexia, and pain.[8] Through selective binding of the COX-2 enzyme, the production of prostaglandins can be reduced, leading to reductions in inflammation and pain.[9] There are multiple NSAIDs currently available for veterinary use, including carprofen, firocoxib, ketoprofen, and meloxicam.[10]

A new NSAID available for veterinary use is robenacoxib (Onsior; Elanco Inc), which was originally developed for the control of inflammation, pain, and hyperthermia in canine and feline patients. Robenacoxib is described as having highly selective and targeted inhibition of the COX-2 enzyme with weak binding and inhibition of COX-1. Furthermore, robenacoxib is highly protein bound, allowing it to be easily transported to sites of inflammation within the body and to remain active at those sites despite a rapid elimination half-life in the blood.[6,11,12] Given the high affinity of robenacoxib for the COX-2 enzyme with minimal COX-1 inhibition, persistence at sites of inflammation, and potential for longer dosing intervals, the use of robenacoxib in elasmobranchs may provide an opportunity to improve analgesic and anti-inflammatory therapy in these species.[13]

Multiple NSAIDs have been previously evaluated in fish, including carprofen, ketoprofen, meloxicam, and robenacoxib, with these studies[5,14] showing variable efficacy in analgesic and anti-inflammatory properties. There are limited data available on the use of NSAIDs in elasmobranchs specifically and prior research has focused on the use of oral and injectable ketoprofen and meloxicam.[15–18] Currently, the only pharmacokinetic study[19] evaluating the use of robenacoxib in a fish species was performed in rainbow trout (*Oncorhynchus mykiss*). Therefore, the objective of this study was to describe the pharmacokinetics of a single intramuscular injection of robenacoxib at 4.0 mg/kg in smooth dogfish (*Mustelus canis*).

Methods

This study was reviewed and approved by the Indianapolis Zoo Research Review Committee. Eight adult male smooth dogfish, weighing 3.1 to 4.25 kg (mean = 3.80; SD = 0.45), between 13 and 16 years of age (mean = 14; SD = 0.78), were maintained in a 10,000-gallon enclosure at the Indianapolis Zoo. Water quality parameters were stable throughout the study. Average parameters included a temperature range of 68 °F to 75 °F, salinity range of 30 to 34 ppt, and pH range of 7.95 to 8.3. Additionally, the ammonia concentration was maintained between 0 and 0.1 mg/L, nitrite ranged from 0 to 0.1 mg/L, and nitrate ranged from 0 to 50 mg/L. The sharks were exposed to cycles of 10 hours of light and 14 hours of dark per day. They were fed a diet consisting of aquaculture shrimp, Pacific herring, capelin, smelt, trout, Atlantic silversides, and squid. The sharks were also each provided a daily supplemental vitamin (Shark & Ray Supplement; Mazuri Exotic Animal Nutrition). At the time of the study, these sharks were housed only with conspecifics and no different species within the enclosure.

Before the initiation of the study, all sharks were determined to be healthy based on visual examination by a veterinarian and comprehensive hematology, plasma biochemistry, and plasma electrophoresis results collected within 1 week of the initiation of the study and compared to established values for this species.[20] A body weight from each shark was obtained within 30 days before the start of the study.

Experimental design

Two initial pilot studies were performed to optimize the efficiency of the main study by determining proper dosing, timing, and collection technique. In the first pilot study, a single dose of robenacoxib at 2.0 mg/kg was administered intramuscularly caudolateral to the dorsal fin and above the lateral line on the right side of the body in 2 sharks. In the second pilot study, a single dose of robenacoxib at 4.0 mg/kg was administered intramuscularly in the same location in 2 additional sharks that were not utilized in the first pilot study. One of the sharks from the first pilot study was sampled again during the main study with a washout period of 11 weeks between studies. The methodology of both pilot studies adhered to that of the main study.

Based on data acquired through the pilot studies, each shark received a single dose of robenacoxib at 4.0 mg/kg, administered using a 22-gauge needle attached to a 1-mL syringe intramuscularly caudolateral to the dorsal fin and above the lateral line on the right side of the body. Manual pressure was applied to the injection site for approximately 5 seconds after administration to minimize drug leakage.

Blood samples were collected under manual restraint from the ventral tail vessel with the animal in ventrodorsal recumbency at 30 minutes, 1 hour, 2 hours, 4 hours, 8 hours, and 24 hours after drug administration. Three sharks were sampled at each time point with no shark being sampled more than twice within a 24-hour time period. The total blood volume collected from each shark was no more than 1% of total body weight. Blood was placed into lithium heparin tubes (MiniCollect; Greiner Bio-One) and centrifuged at 1,030 X *g* for 5 minutes within 1 hour of collection, and plasma was collected and stored in plastic cryovials at −80 °C until analysis. All sharks were monitored daily following drug administration and venipuncture to evaluate for any adverse effects of handling, treatment, and sampling.

Robenacoxib quantification

Validation of the HPLC procedure for robenacoxib followed the guidelines of bioanalytical method validation established by the FDA. Plasma samples were analyzed using a reverse phase HPLC method. The system consisted of a 2695 separations module and a 2487 absorbance detector. The compounds were separated on a Sunfire C_{18} (4.6 X 150 mm, 3.5 µm) column with a Sunfire C_{18} guard column. The mobile phase was a mixture of 0.025% trifluroacetic acid and acetonitrile (50:50). The flow rate was 1.1 mL/min, and absorbance was measured at 275 nm.

Robenacoxib was extracted from plasma samples using liquid-liquid extraction. Frozen plasma samples were thawed and vortexed, and 100 µL was transferred to a screw-top test tube followed by 25 µL internal standard (1.0 µg/mL deracoxib). Methylene chloride (4 mL) was added, and the tubes were vortexed for 60 seconds and then centrifuged

for 15 minutes at 1,000 X *g*. The organic layer was transferred to a glass tube and evaporated to dryness with nitrogen gas. Samples were reconstituted in 200 µL of mobile phase, and 100 µL was analyzed.

Standard curves for plasma analysis were prepared by fortifying untreated, pooled plasma with robenacoxib to produce a linear concentration range of 0.1 to 100 µg/mL. Calibration samples were prepared exactly as plasma samples. Average recovery for robenacoxib was 95%, while intra- and interassay variability ranged from 4.9 to 9.2% and 2.2 to 10.9%, respectively. The lower limit of quantification was 0.1 µg/mL. The lower limit of detection was 0.05 µg/mL.

Robenacoxib analysis

A noncompartmental analysis of the time-concentration profile was performed using the sparse sampling option in Phoenix (Certara). This option calculates the pharmacokinetic parameters from the mean curve of the pooled data. Parameters for this analysis are the maximum observed plasma concentration (C_{max}), time to the maximum observed plasma concentration, and the slope of terminal portion of the time-concentration profile (elimination rate constant) and elimination half-life. AUC was calculated using the trapezoidal rule and extrapolated to infinity.

Results

One of the sharks sampled in the second pilot study was found to be minimally responsive in its habitat 3 days after drug administration. Medical care was provided, but ultimately, humane euthanasia was performed. Histologic examination of tissues collected postmortem revealed that the probable cause of death was sepsis with evidence of infection and inflammatory changes in multiple organs. Per the pathologist, none of the lesions correlated with an adverse drug reaction. Three sharks showed evidence of mild bruising at or near the injection site shortly after drug administration during the main study, which resolved within 8 hours. Throughout and following this study all other sharks remained clinically normal. Results of the noncompartmental analysis are described **(Table 1)**, and mean (± SD) plasma concentrations are depicted **(Figure 1)**.

Table 1—Pharmacokinetic parameters of a single intramuscular dose of robenacoxib (4.0 mg/kg) in the smooth dogfish (*Mustelus canis*).

Pharmacokinetic parameter	Robenacoxib
$t_{½}$ (h)	3.79
k_{el} (1/h)	0.18
t_{max} (h)	0.5
C_{max} (µg/mL)	1.24
$AUC_{0\text{-last}}$ (h·µg/mL)	4.27
$AUC_{0\text{-}\infty}$ (h·µg/mL)	5.52

$AUC_{0\text{-}\infty}$ = Area under the plasma concentration time curve from time 0 to infinity. $AUC_{0\text{-last}}$ = Area under the plasma concentration time curve from time 0 to last point. C_{max} = Maximum plasma concentration. k_{el} = Elimination rate constant. $t_{½}$ = Elimination half-life. t_{max} = Time to maximum plasma concentration.

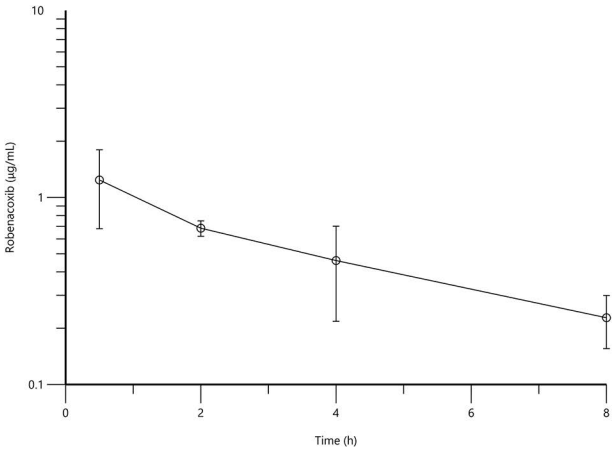

Figure 1—Mean (± SD) plasma concentrations of robenacoxib in the smooth dogfish after administration of a single intramuscular dose (4.0 mg/kg).

Discussion

To the author's knowledge, this is the first study to evaluate the use of robenacoxib in any elasmobranch species. The results of this study suggest that robenacoxib exhibited rapid absorption and elimination in smooth dogfish, with minimal side effects noted after administration. Furthermore, the C_{max} was substantially higher than what is considered to be therapeutic in domestic species (0.0141 µg/mL) and was maintained above this concentration for at least 8 hours.[21]

Comparing the pharmacokinetic results in this study to those obtained in a study of robenacoxib in rainbow trout, a shorter mean time to maximum plasma concentration (0.5 hours vs 2.1 hours) and a shorter mean terminal half-life (3.79 hours vs 12.61 hours) was observed in smooth dogfish. Furthermore, a substantially higher C_{max} was achieved in smooth dogfish compared to the rainbow trout (1.24 µg/mL vs 0.524 µg/mL). However, this is not surprising given that a higher dosage of robenacoxib (4.0 mg/kg) was used in the smooth dogfish compared to the dose used in the rainbow trout (2.0 mg/kg).

This study focused on the use of the injectable formulation of robenacoxib; however, the use of an oral formulation of this medication in elasmobranchs warrants further investigation to determine the optimal route of administration. A previous study[16] comparing the pharmacokinetics of intramuscular versus oral administration of meloxicam, a similar NSAID, in yellow stingrays (*Urobatis jamaicensis*) showed that oral administration maintained therapeutic plasma concentrations for substantially longer than intramuscular administration.

There are numerous factors that need to be considered when evaluating the results of this study. The study population consisted of a small number of animals and was composed entirely of males. Therefore, any sex-based differences in metabolism may alter recommendations for dosage amount and frequency in this species. Furthermore, the study population was composed of geriatric sharks that

were beyond the average life expectancy for this species. As such, age-related alterations in drug metabolism cannot be ruled out. Despite these limitations, these results provide preliminary evidence for the use of robenacoxib in elasmobranch species. To be complete, future studies should also focus on the pharmacodynamic effects of robenacoxib in this species. A sparse sampling method was employed in this study, chosen to decrease the stress of handling and venipuncture on each individual shark. However, this sampling method does preclude the ability to evaluate interindividual variability both within and between the sharks used in this study. Finally, as robenacoxib is noted to remain at sites of inflammation longer than within the blood, our results may also underestimate the actual robenacoxib activity at sites of inflammation.[6]

In conclusion, this study suggests that intramuscular use of robenacoxib (4.0 mg/kg) in smooth dogfish results in rapid absorption and plasma concentrations above levels considered therapeutic in domestic species without adverse effects. However, a more frequent dosing interval may be required due to relatively rapid plasma elimination.

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Disclosures

The authors have nothing to disclose. No AI-assisted technologies were used in the generation of this manuscript.

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References

1. Garner MM. A retrospective study of disease in elasmobranchs. *Vet Pathol.* 2013;50(3):377–389. doi:10.1177/0300985813482147
2. Janse M, Zimmerman B, Geerlings L, Brown C, Nagelkerke LAJ. Sustainable species management of the elasmobranch populations within European aquariums: a conservation challenge. *J Zoo Aquarium Res.* 2017;5(4):172–181. doi:10.19227/jzar.v5i4.313.
3. Smith M, Warmolts D, Thoney D, Hueter R, eds. *The Elasmobranch Husbandry Manual: Captive Care of Sharks, Rays and Their Relatives*. Ohio Biological Survey; 2004:589.
4. Chatigny F, Creighton CM, Stevens ED. Updated review of fish analgesia. *J Am Assoc Lab Anim Sci.* 2018;57(1):5–12.
5. Neiffer DL. Chapter 10: Anesthesia and analgesia. In: Hadfield CA, Clayton LA, eds. *Clinical Guide to Fish Medicine.* 1st ed. Wiley; 2021:198–212. doi:10.1002/9781119259886.
6. Kongara K, Chambers P. Robenacoxib in the treatment of pain in cats and dogs: safety, efficacy, and place in therapy. *Vet Med (Auckl).* 2018; 9:53–61. doi:10.2147/VMRR.S170893
7. Rao P, Knaus EE. Evolution of nonsteroidal anti-inflammatory drugs (NSAIDs): cyclooxygenase (COX) inhibition and beyond. *J Pharm Pharm Sci.* 2008;11(2):81. doi:10.18433/J3T886
8. Flower RJ. The development of COX2 inhibitors. *Nat Rev Drug Discov.* 2003;2(3):179–191. doi:10.1038/nrd1034
9. Curry SL, Cogar SM, Cook JL. Nonsteroidal anti-inflammatory drugs: a review. *J Am Anim Hosp Assoc.* 2005;41(5):298–309. doi:10.5326/0410298
10. Plumb DC. *Plumb's Veterinary Drug Handbook.* 9th ed. Pharma Vet Inc; 2018.
11. Jung M, Lees P, Seewald W, King JN. Analytical determination and pharmacokinetics of robenacoxib in the dog. *J Vet Pharmacol Ther.* 2009;32(1):41–48. doi:10.1111/j.1365-2885.2008.01035.x
12. King JN, Dawson J, Esser RE, et al. Preclinical pharmacology of robenacoxib: a novel selective inhibitor of cyclooxygenase-2. *J Vet Pharmacol Ther.* 2009;32(1):1–17. doi:10.1111/j.1365-2885.2008.00962.x
13. Kamata M, King JN, Seewald W, Sakakibara N, Yamashita K, Nishimura R. Comparison of injectable robenacoxib versus meloxicam for peri-operative use in cats: results of a randomised clinical trial. *Vet J.* 2012;193(1):114–118. doi:10.1016/j.tvjl.2011.11.026
14. Vergneau-Grosset C, Lair S. Chapter 12: Medical treatment. In: Hadfield CA, Clayton LA, eds. *Clinical Guide to Fish Medicine.* 1st ed. Wiley; 2021:233–266. doi:10.1002/9781119259886.
15. Davis MR, Mylniczenko N, Storms T, Raymond F, Dunn JL. Evaluation of intramuscular ketoprofen and butorphanol as analgesics in chain dogfish (*Scyliorhinus retifer*). *Zoo Biol.* 2006;25(6):491–500. doi:10.1002/zoo.20105
16. Kane LP, O'Connor MR, Papich MG. Pharmacokinetics of a single dose of intramuscular and oral meloxicam in yellow stingrays (*Urobatis jamaicensis*). *J Zoo Wildl Med.* 2022;53(1):153–158. doi:10.1638/2021-0123
17. Morón-Elorza P, Rojo-Solís C, Álvaro-Álvarez T, Valls-Torres M, García-Párraga D, Encinas T. Pharmacokinetic studies in elasmobranchs: meloxicam administered at 0.5 mg/kg using intravenous, intramuscular, and oral routes to nursehound sharks (*Scyliorhinus stellaris*). *Front Vet Sci.* 2022;9:845555. doi:10.3389/fvets.2022.845555
18. Morón-Elorza P, Cañizares-Cooz D, Rojo-Solis C, et al. Pharmacokinetics of the anti-inflammatory drug meloxicam after single 1.5 mg/kg intramuscular administration to undulate skates (*Raja undulata*). *Vet Sci.* 2022;9(5):216. doi:10.3390/vetsci9050216
19. Raulic J, Beaudry F, Beauchamp G, et al. Pharmacokinetic, pharmacodynamic, and toxicology study of robenacoxib in rainbow trout (*Oncorhynchus mykiss*). *J Zoo Wildl Med.* 2021;52(2):529–537. doi:10.1638/2020-0130
20. Persky ME, Williams JJ, Burks RE, Bowman MR, Ramer JC, Proudfoot JS. Hematologic, plasma biochemistry, and select nutrient values in captive smooth dogfish (*Mustelus canis*). *J Zoo Wildl Med.* 2012;43(4):842–851. doi:10.1638/2012-0002R1.1
21. Pelligand L, King JN, Toutain PL, Elliott J, Lees P. Pharmacokinetic/pharmacodynamic modelling of robenacoxib in a feline tissue cage model of inflammation: robenacoxib PK/PD modelling in the cat. *J Vet Pharmacol Ther.* 2012;35(1):19–32. doi:10.1111/j.1365-2885.2011.01288.x