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PHARMACOKINETICS OF SINGLE DOSE ORAL MELOXICAM IN BOTTLENOSE DOLPHINS (*TURSIOPS TRUNCATUS*)

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Abstract: The objective of this study was to investigate the pharmacokinetics of meloxicam in bottlenose dolphins (*Tursiops truncatus*). Ten adult bottlenose dolphins were used for the study. Each animal received a single oral dose of meloxicam at 0.1 mg/kg. Two to seven serial blood samples were collected per animal, at one of fourteen time points between T = 0 and T = 240 hr. Complete blood count and serum chemistry analysis were performed prior to drug administration, as well as at the final time point for each individual. Plasma drug concentrations were determined by high-pressure liquid chromatography. No adverse hematological, biochemical or clinical changes were noted during the study period. After oral administration, a peak plasma concentration of 1.03 µg/mL was achieved at approximately 11 hr. This suggests that a single oral dose of 0.1 mg/kg provides a peak plasma level similar to what is considered therapeutic in other species. However, the elimination of meloxicam in cetaceans was slower than in other species, with an elimination half-life of almost 70 hr, and detectable drug concentrations up to 7 days. A single oral dose of 0.1 mg/kg appears safe for use in this species, but caution in repeated dosing must be used, due to the prolonged elimination, until multi-dose pharmacokinetic studies are determined.

Key words: Bottlenose dolphin, pharmacokinetics, meloxicam, *Tursiops truncatus*.

INTRODUCTION

Nonsteroidal anti-inflammatory drugs (NSAIDs) are commonly used to treat both acute and chronic pain and inflammation. A variety of non-steroidal drugs have been administered to cetacean species, including flunixin meglumine, acetaminophen, carprofen, celecoxib, and meloxicam, among others.^{9,12} Use of NSAIDs is limited in cetaceans due in part to both unpublished accounts and scientific publications reporting adverse effects, especially gastrointestinal side effects. A case of fatal perforation of the connecting channel of the gastrointestinal tract following administration of flunixin meglumine has been reported.¹² The authors have also experienced

additional cetacean cases with gastrointestinal ulceration, hemorrhage, and perforations associated with extended use of NSAIDs for greater than 1 wk.

Very few pharmacokinetics studies have been performed in cetaceans to date, and at present, there are no published pharmacokinetics studies of any NSAIDs. The authors hypothesize that cetaceans have pharmacokinetic differences that significantly alter metabolism and reduce clearance of this class of drugs, and that the dose ranges currently used are inappropriate. Despite the lack of widespread use of NSAIDs in cetacean medicine, NSAIDs have the potential to be of great use in reducing both acute and chronic inflammation.

Meloxicam is an enolic acid NSAID of the oxicam group. It has been used extensively in humans and companion animals. Unlike many other NSAIDs, meloxicam has high oral availability and a long half-life, which makes it an attractive drug for veterinary use.^{1,2,6} This drug was also chosen due to promising anecdotal efficacy with no observed clinical adverse effects when used by the co-authors on a small number of cetaceans. In terrestrial mammals, meloxicam is primarily cleared via the liver, and it displays a high degree of plasma protein binding.⁸ While less is known about the hepatic physiology of the dolphin, it is hypothesized that meloxicam should exhibit similar binding and clearance in this

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species. The purpose of this study was to investigate the uptake and pharmacokinetics of meloxicam when administered as a single oral dose in bottlenose dolphins.

MATERIALS AND METHODS

Animals and housing

Ten adult bottlenose dolphins (*Tursiops truncatus*), six females and four males, ranging in age from 12–23 years were used for this study. Body weights ranged between 178–240 kg. The total dose calculation was based on a body weight taken within seven days of the study.

The dolphins were either housed in open-ocean enclosures at the U.S. Navy Marine Mammal Program in San Diego, California ($n = 2$) or in above-ground pools with natural sea water at SeaWorld in San Diego, California ($n = 8$). All dolphins were fed their previously established diet of frozen-thawed whole fish, consisting of capelin (*Mallotus villosus*), Pacific herring (*Clupea harengus*), Atka mackerel (*Pleurogrammus azonus*), sardine (*Sardina pilchardus*), and market squid (*Loligo opalescens*) throughout the course of the study.

Physical exams were performed on all dolphins within 48 hr prior to study commencement, and all study subjects were clinically normal based on the exams. Four animals were receiving prophylactic medications at the time of the study. None of the medications or medical conditions were likely to interfere with organ function responsible for metabolism and excretion of the drug. No medications were administered at the time of meloxicam dosing so as not to interfere with absorption.

Experimental design

Each dolphin received a single oral dose of meloxicam (Meloxicam tablets, Mylan Pharmaceuticals, Morgantown, West Virginia 26505, USA) at 0.1 mg/kg, rounded to the nearest one-fourth tablet, or 3.75 mg, resulting in an average dose of 0.103 mg/kg. The 0.1 mg/kg dose is the widely accepted daily dose for dogs,¹ and was chosen based on the authors' experience with the dose, and the desire to perform the pilot study with a conservative dose to assess plasma concentrations. Animals were fasted overnight. Tablets were placed inside a capelin, and administered following animal acceptance of a non-medicated capelin to reduce the chance of medication rejection, which is a common method of medication administration in this species. Normal feeding schedules were resumed one hour following medication administration.

An initial pilot study was performed using two dolphins for meloxicam plasma level assessment and to confirm meloxicam uptake and dosage. Animals presented for voluntary blood collection at $T = 1, 2, 4, 8, 12, 24,$ and 48 hr. Approximately 10 ml of whole blood was collected in sodium heparin tubes from the peri-arterial venous rete on the ventral fluke blade using a 23 G needle. Samples were centrifuged within 10 minutes of collection. Plasma was then pipetted into cryovials and frozen at -80°C until analysis.

Following the pilot study, a second study was conducted to determine the pharmacokinetics of meloxicam. A total of 22 blood samples were drawn throughout a 240-hr time period. Dolphins ($n = 8$) were randomly assigned into groups for blood collection time points. A blood sample was drawn from each dolphin immediately prior to meloxicam administration ($T=0$), and then two to three additional samples were drawn over a 240-hr time period. All data were then pooled to achieve at least $n=2$ for the time points $T=0, 1, 2, 4, 6, 8, 10, 12, 24, 48, 72, 96, 168, 240$ hr. Data from both the pilot study and clinical trial were pooled and results are discussed below.

All animals in both the pilot and follow-up study had blood collected prior to meloxicam administration, as well as at the final time point for that individual, to assess clinical hematology values. The blood was collected in serum and $\text{K}_2\text{-EDTA}$ tubes, and submitted within one hour for complete blood count (CBC) and serum chemistry analysis, evaluating 40 hematological and biochemical parameters. Plasma was also pooled from the initial blood draw and used as a control (blank) for the pharmacokinetic assay.

A paired T-test was used to compare mean hematological values before treatment (baseline) versus after treatment. Statistical significance was set *a priori* at values of $P < 0.05$. All statistical analyses were performed using SAS software, version 9.1 (PROC T Test, SAS Institute, Inc., Cary, North Carolina 27513, USA).

Plasma drug analysis

Plasma concentrations of meloxicam were determined using high-pressure liquid chromatography (HPLC) in the Clinical Pharmacology Laboratory at North Carolina State University.

Reference standards for meloxicam were purchased from Sigma-Aldrich (Meloxicam United States Pharmacopeia Reference Standard, Sigma-Aldrich Corporation, St. Louis, Missouri 63101). Meloxicam reference standard was weighed and dissolved in a mixture of methanol and 1 N

sodium hydroxide (50:3) to a concentration of 1 mg/mL. From this stock solution, further dilutions were made in HPLC grade distilled water to make up fortifying solutions for plasma in order to prepare quality control samples, calibration curve samples, and for development of these methods. The stock solution was kept at 4°C in a tightly sealed dark vial. The fortifying solutions made from the stock solution were added to blank (control) plasma, to make up seven calibration standards, including zero (range 0.0 µg/mL to 10 µg/mL).

The mobile phase for HPLC analysis consisted of 40% acetonitrile, and 60% 0.05 M sodium acetate buffer. Glacial acetic acid was added to the buffer to adjust the pH to 3.7 to 3.8. Fresh mobile phase was prepared, filtered (0.45 µm), and degassed for each day's run.

The HPLC system consisted of a quaternary solvent delivery system at a flow rate of 1 ml/min, an autosampler, and UV detector set at a wavelength of 365 nm (1260 Series Autosampler, Agilent Technologies, Santa Clara, California 95051, USA). The chromatograms were integrated with a computer program (1260 Series Chemstation software, Agilent Technologies, Santa Clara, California 95051, USA). The column was a reverse-phase, 4.6 mm × 15 cm C8 column (Agilent Technologies, Inc., Santa Clara, California 95051, USA) kept at a constant temperature of 40°C. Retention time for the peak of interest was approximately 2.48–2.49 min.

All plasma samples, calibration samples, and blank (control) plasma samples were prepared identically using solid-phase extraction. Solid phase extraction cartridges (Waters Oasis HLB cartridges, Waters Associates, Millford, Massachusetts 01757, USA) were conditioned with 1 ml methanol followed by 1 ml distilled water. A plasma sample of 250 µL was added to a conditioned cartridge, followed by a wash step of 1 ml distilled water: methanol (95:5). The drug was eluted with 1 ml 100% methanol and collected in clean glass tubes. The tubes were evaporated at 40°C for 15–20 min in an evaporator. Each tube was then reconstituted with 200 µL of mobile phase and vortexed. From each tube, 30 µL was then injected into the HPLC system. A fresh set of calibration and blank samples were prepared for each day's run. All calibration curves were linear with a R² value of 0.99 or higher. Limit of quantification for this study was 0.05 µg/mL, which was determined from the lowest point on a linear calibration curve that met our acceptance criteria and using guidelines published by the

United States Pharmacopeia (General Chapter 1225, United States Pharmacopeia, Rockville, Maryland 20851, USA).

Pharmacokinetic analysis

Because of the sparse sampling from these dolphins, data density from individuals was not sufficient for a traditional standard two-stage pharmacokinetic approach. Such an analysis would have required more samples per individual animal, which was not feasible. Instead, the investigators performed a naïve pooled data analysis of samples. In this analysis, samples are pooled across time points, and a single pharmacokinetic analysis performed. The advantage of this analysis is that it allows for sparse sampling in species difficult to handle or sample, but the disadvantage is that it provides no measure of variability in pharmacokinetic parameters across the animals in the study.

Plasma drug concentrations were plotted on linear and semilogarithmic graphs for analysis and visual assessment of the best model for pharmacokinetic analysis. Analysis of curves and pharmacokinetic modeling were then performed by use of a commercial pharmacokinetic program (Phoenix software, Pharsight, Inc., St. Louis, Missouri 63132, USA). Compartmental analysis was performed using a weighting factor of 1/(predicted Y²), where Y is the plasma concentration. The model selected based on best fit was a one-compartment open model with first order absorption, using the following formula:

$$C = \frac{k_{01} F D}{V(k_{01} - k_{10})} [e^{-k_{10}t} - e^{-k_{01}t}],$$

where C is the plasma concentration, t is time, k₀₁ is the non-intravenous (IV) absorption rate constant, assuming first-order absorption, k₁₀ is the elimination rate constant, V is the apparent volume of distribution, F is the fraction of drug absorbed, and D is the non-IV dose. In this model, it is assumed that k₀₁ >>> k₁₀, or that there is no "flip-flop" effect caused by slow absorption from the gastrointestinal tract. Secondary parameters from the model included the peak plasma concentration (C_{MAX}), time of peak concentration (T_{MAX}), area under the plasma-concentration versus time profile (AUC), and the respective absorption and terminal half-life (t_{1/2}).

RESULTS

No adverse clinical effects were noted during the study period of up to 10 days following initial

Table 1. Pharmacokinetic parameters for meloxicam (0.1 mg/kg) in bottlenose dolphins ($n = 10$) following oral administration.

Parameter	Estimate
VD/F (mL/kg) ^a	88.95
K ₀₁ (1/h) ^b	0.32
K ₁₀ (1/h) ^c	0.01
AUC (h* μ g/mL) ^d	115.87
Half-life absorption (hr)	2.19
Half-life elimination (hr)	69.36
CL/F (mL/h/kg) ^e	0.89
T _{MAX} (hr) ^f	11.27
C _{MAX} (μ g/mL) ^g	1.03

^a VD/F apparent volume of distribution per fraction absorbed.

^b K₀₁ absorption rate constant.

^c K₁₀ elimination rate constant.

^d AUC area under the plasma-concentration versus time profile.

^e CL/F systemic clearance per fraction absorbed.

^f T_{MAX} time of peak concentration.

^g C_{MAX} peak plasma concentration.

administration. No animals showed evidence of anemia, azotemia, hepatic or GI disturbances. No significant differences were detected between the 40 CBC and serum chemistry parameters prior to and following drug administration. The mean pharmacokinetic parameters of meloxicam in dolphins following oral administration at 0.1 mg/kg are shown in Table 1 and Figure 1. A C_{MAX} of 1.03 μ g/ml was recorded at 11.27 hr following oral administration. This was associated with a mean plasma t 1/2 of 69.4 hr. During the analysis there were two late terminal time points at 240 and 242 hr that skewed the elimination phase of the curve if these were left in the analysis (t 1/2 with these points retained was 135 hr). Because these points were deemed to be outliers, which could not be confirmed with re-analysis or from other animals, and there were no points between 168 and 240 hr to verify the slope of the curve during that phase, these two points were eliminated from the final analysis.

DISCUSSION

Pharmacokinetic studies in wildlife species that are difficult to either handle or sample are common in scientific literature, and allow for evaluation of drug parameters with fewer samples than in traditional clinical trials.^{4,7} However, a small sample size does provide uncertainty when outliers are presented. In this study, several pharmacokinetic parameters were surprising in comparison with other species' results and dosing

recommendations. The elimination t 1/2 of almost 70 hr found in this study is longer than reports in other species, although it is known that meloxicam metabolism can vary greatly among species (Table 2). When given at similar oral doses, the half-life is as short as 6 hr in baboons, and as long as 24 hr in dogs and humans.^{2,11} A higher dose of 1 mg/kg in rats produced a t 1/2 of 49.9 hr, the most similar value to dolphins.² T_{MAX} values also vary widely across species, ranging from 0.7 hr in mice, to 7.5 hr in dogs.²

A previous study of the role of meloxicam in reducing iatrogenic joint inflammation in horses suggested that meloxicam plasma concentrations as low as 0.2 μ g/ml can have anti-inflammatory effects.¹⁰ The target therapeutic range is not known in dolphins, but based on equine observations and extrapolating to dolphins, therapeutic levels extended up to 7 days in this study, based on the data up to 168 hr. As the meloxicam dosing interval in other species is once daily, one hypothesis for explaining adverse effects of NSAIDs that has been noted in dolphins is that prolonged plasma concentrations may lead to accumulation when administered daily.

While the drug concentrations reported fall within the range of concentrations that have been deemed therapeutic in other species, these data are extrapolating a clinical effect. Accounts of several of the authors have suggested a positive clinical effect when using meloxicam at this dose for acute injuries. The inflammatory cascade is poorly understood in cetaceans, and although they are believed to have the same basic mammalian system of cyclooxygenases, the role specific mediators play in the inflammatory cascade remains to be studied.

Meloxicam has the potential to be a very useful tool in managing inflammation and pain in dolphins. Managing side effects, namely gastrointestinal injury, is of greatest concern, due to frequent reports of gastrointestinal upset in this species. The dolphin stomach is multi-chambered, consisting of a non-glandular forestomach, glandular fundic chamber, connecting channel, and pyloric chamber.⁵ While endoscopically visible ulcerations of the forestomach may be found in clinically ill cetaceans, ulceration and fatal perforation of the gastrointestinal tract following glucocorticoid or NSAID use has been reported in the connecting channel, which is not accessible via endoscopy.¹² Hence, gastroscopies were not included as monitoring tool for adverse side effects in this study.

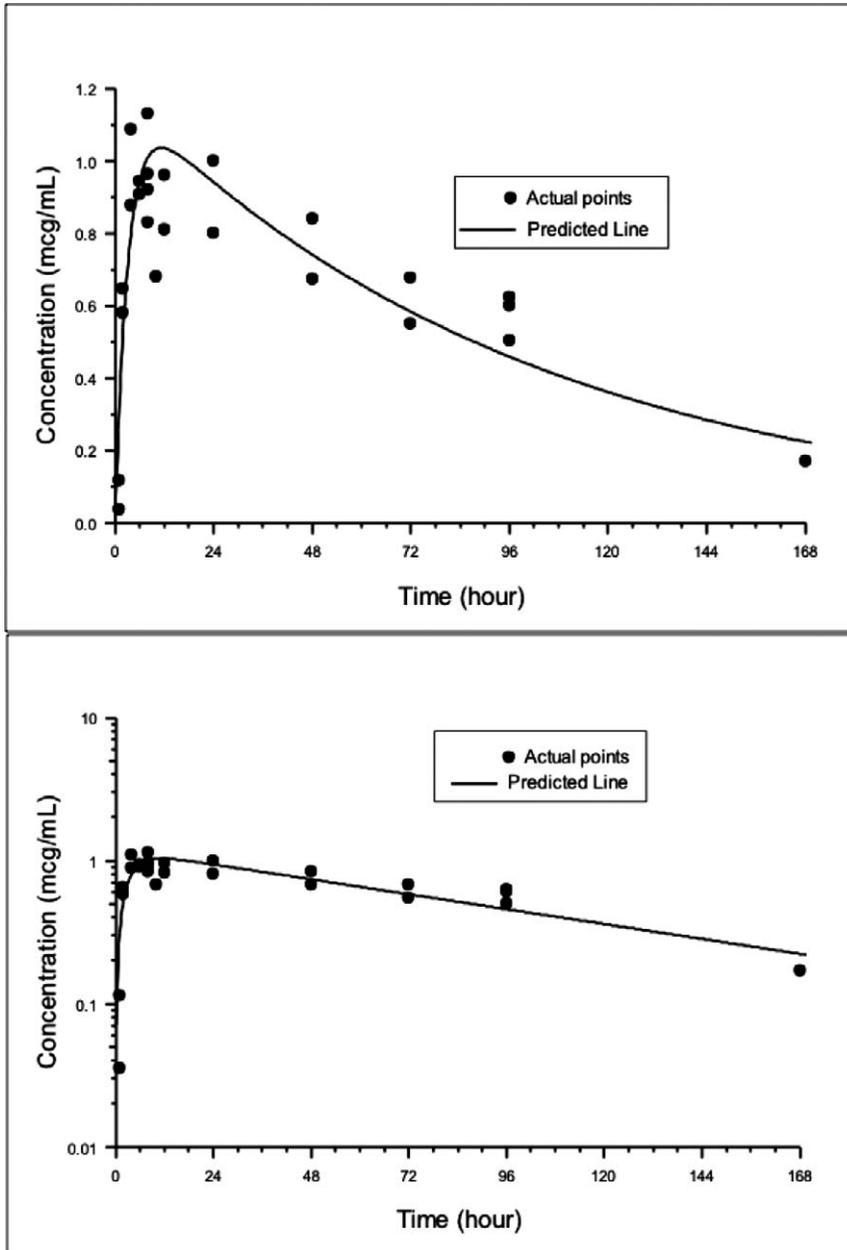


Figure 1. Mean plasma concentration ($\mu\text{g}/\text{ml}$) of meloxicam in bottlenose dolphins ($n = 10$) following oral administration of a single dose ($0.1 \text{ mg}/\text{kg}$). Solid points represent individual concentrations, some of which are overlapping, and the solid line represents the fitted line from the pharmacokinetic model. Top panel is represented on a linear axis, bottom panel is represented on a semi logarithmic axis.

Acute renal damage has not been documented with NSAID use in cetaceans. Moderate renal impairment did not change the pharmacokinetic profile of meloxicam in humans.¹¹ These findings are consistent with this study's results, which saw no changes in either blood urea nitrogen or creatinine.

Meloxicam administration was standardized in this study to be given inside a single capelin with a second capelin given as a follow-up to reduce variability of absorption. Although meloxicam has a nearly 100% bioavailability in dogs when administered with food per the manufacturer's instructions, a study in horses showed that, while

Table 2. Meloxicam dosing, peak plasma concentration, and half-life reported for multiple species following oral administration of meloxicam.

Species	Dosage (mg/kg)	C _{MAX} (µg/ml) ^a	t 1/2 (hours) ^b
Bottlenose dolphin	0.1	1.03	69.4
Dog ²	0.2	0.47	23.7
Rat ²	1	2.35	49.9
Llama ⁶	1	1.31	22.7
Rabbit ³	0.2	0.17	8.3
Human ¹¹	0.2	0.90	23.5
Horse ¹³	0.6	1.58	5.0

^a C_{MAX} peak plasma concentration.

^b t 1/2 plasma half-life.

bioavailability is not changed with feeding, there is a greatly slowed rate of drug absorption when administered with hay.^{1,10} Most captive dolphin populations have a diet consisting of varying ratios of a few species of fish, but varied meal sizes could potentially alter absorption.

This study was not able to determine the absolute oral absorption of meloxicam because there was not an accompanying IV study to compare. The values of T_{MAX} and C_{MAX} cannot be used to assess bioavailability. Peak plasma concentrations attained were higher than the target therapeutic concentration of 0.2 µg/ml described in other species¹⁰. The drug appears to have a longer half-life than in other species studied (Table 2), and in order to decrease the risk of adverse effects, a longer dosing interval than daily dosing is likely required. However, further studies are required to determine the optimal dosing interval. Based on these results, we must caution against repeated dosing, as the drug circulates for much longer than previously known. A single oral dose of meloxicam at 0.1 mg/kg appears to be safe for treatment of acute inflammation in this species.

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