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PHARMACOKINETICS OF TRAMADOL HYDROCHLORIDE AND ITS METABOLITE O-DESMETHYLTRAMADOL FOLLOWING A SINGLE, ORALLY ADMINISTERED DOSE IN CALIFORNIA SEA LIONS (*ZALOPHUS CALIFORNIANUS*)

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Abstract: Tramadol is a synthetic, centrally acting, opiate-like analgesic that is structurally related to codeine and morphine. The objective of this study was to determine the pharmacokinetics of tramadol hydrochloride and its major active metabolite O-desmethyltramadol (M1) in the California sea lion (*Zalophus californianus*). A single dose of tramadol was administered orally in fish at 2 mg/kg to a total of 15 wild California sea lions admitted for rehabilitation. Twenty-four total blood samples were collected post drug administration at 10, 20, 30, and 45 min and at 1, 3, 5, 6, 8, 12, and 24 hr. Blood plasma was separated and stored at −80°C until analysis with high-performance liquid chromatography was performed to determine levels of tramadol and M1, the major active metabolite. The results indicate that the plasma levels of parent tramadol are low or negligible during the first 30–45 min and then reach the predicted mean maximum plasma concentration of 358 ng/ml at 1.52 hr. The M1 metabolite was not detectable in 21 of 24 plasma samples, below the level of quantification of 5 ng/ml in one sample, and detectable at 11 and 17 ng/ml in two of the samples. This study suggests that a 2 mg/kg dose would need to be administered every 6–8 hr to maintain concentrations of tramadol above the minimum human analgesic level for mild to moderate pain. Based on dosing simulations, a dose of 4 mg/kg q8 hr or q12 hr, on average, may represent an adequate compromise, but further studies are needed using a larger sample size. Pharmacodynamic studies are warranted to determine if tramadol provides analgesic effects in this species. The potential for tramadol toxicosis at any dose also has not been determined in this species.

Key words: California sea lion, M1, marine mammal, tramadol, *Zalophus californianus.*

INTRODUCTION

California sea lions (*Zalophus californianus*), both wild and in captivity, may present for conditions in which pain control medications are warranted. Many sea lions admitted to rehabilitation facilities have traumatic injuries such as shark bites, gunshot wounds, or other conditions requiring surgical intervention. Therefore, having an analgesic agent available that is safe, orally bioavailable, and long-lasting would be advantageous.

Tramadol is a synthetic, centrally acting, opiate-like analgesic structurally related to codeine and morphine active at the μ, κ, and δ opioid receptors, with selectivity for the μ-receptor.[20] It also inhibits the monoaminergic pathway, which is responsible for serotonin (5HT) and norepinephrine reuptake, and enhances the inhibitory effect of pain transmission in the spinal cord.[14] Tramadol has a wide safety margin in humans, without the respiratory depression, constipation, or sedation that other opioids cause, as well as having minimal cardiovascular effects.[2,8,19] Tramadol is an appealing analgesic drug for California sea lions as it can be administered orally by placing it into fish, it has a high oral bioavailability, and its metabolism is not affected when administered with food.[12]

Tramadol is metabolized primarily by the P-450 enzyme system in the liver into several metabolites, with the major active metabolite being O-desmethyltramadol (M1).[19,28] M1 is the main analgesic effective metabolite, having two to 200 times more potency in binding to μ-receptors than does parent tramadol.[20] In lab animal studies, M1 was shown to be six times more potent an analgesic than parent tramadol.[17] Other metabolites that are produced, including N-desmethyl-

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tramadol (M2), N,N-didesmethyltramadol (M3), N,N,O-tridesmethyltramadol (M4), and N,O-didesmethyltramadol (M5), have not been shown to produce analgesia in mammals.[28]

Tramadol is a commonly used analgesic in human and small animal medicine. Pharmacokinetic studies evaluating tramadol have been carried out in numerous species including cats, dogs, rats, horses, goats, rabbits, and several avian species.[2,4–7,9–15,18,20–24,26–28] In dogs, pharmacokinetic studies combined with antinociceptive studies have been done in several canine breeds using different routes of drug administration. Studies in beagles have shown tramadol to be an effective analgesic when given intravenously prior to abdominal and orthopedic surgery and when given intravenously or subcutaneously postoperatively following ovariohysterectomy.[4,13] Antinociceptive effects have also been documented following oral administration of tramadol in greyhounds.[11]

The objective of this study was to determine the pharmacokinetic parameters of orally administered tramadol and its metabolite M1 in California sea lions (*Zalophus californianus*) admitted for rehabilitation to The Marine Mammal Center (TMMC) in Sausalito, California to determine if blood plasma levels reached those considered to be analgesic levels in humans and other mammals.

MATERIALS AND METHODS

Study animals and experimental design

Fifteen California sea lions, presented to TMMC in Sausalito, California for rehabilitation under a Standing Agreement from the National Marine Fisheries Service, were included in this study. Individuals in this study were considered healthy by TMMC veterinary staff based on weight gain during rehabilitation, demonstration of ability to consume fish on their own, and completion of treatment for any illness for which they had initially presented. Animals included one pup, nine yearlings, one juvenile, one subadult, and three adults (ten females and five males) ranging in weight from 20.5 to 107.5 kg. All individuals were housed in fenced pens with access to a pool containing salt water and were fed herring at 0800, 1400, and 2200 hours. This study was approved by The Marine Mammal Center Institutional Animal Care and Use Committee.

Tramadol hydrochloride (50 mg tablet, Amneal Pharmaceuticals, Hauppage, New York 11788, USA) was administered orally in a herring at 2 mg/kg to each individual. Doses were calculated based on a current weight within 5 days prior to tramadol administration. Tablets (whole, halved, or quartered as needed to meet dosage requirements) were placed under the operculum of a single thawed herring (approximately 62.5 mg/fish) and administered to individuals following an approximately 10-hr fast. Individuals were visually monitored to confirm fish ingestion, and the remainder of each animal's feed was given thereafter.

Twenty-four total blood samples were collected post drug administration at 10, 20, 30, and 45 min and at 1, 3, 5, 6, 8, 12, and 24 hr. Blood samples were collected from either the right or left caudal gluteal vein using manual restraint and a 20-g, 1–1.5-in needle on a vacutainer set. The samples were placed into ethylenediaminetetraacetic acid blood collection tubes, centrifuged for 10 min, and plasma was removed and placed into a 1.2-ml cryovial. Plasma samples were kept frozen at −80°C until analysis. Sea lions were visually monitored for any adverse reactions to oral tramadol for at least 48 hr following dosing.

Plasma analysis

Analysis of tramadol in plasma samples was conducted using reversed phase high performance liquid chromatography (HPLC) using a method previously published and validated by the laboratory.[29] The system consisted of a 2695 separations module and a 2475 fluorescence detector (Waters, Milford, Massachusetts 01757, USA). Separation was attained on a Waters Symmetry C_{18} 4.6 × 250 mm (5 μm) preceded by a 5 μm Symmetry guard column. The mobile phase was a mixture of (A) 0.01M potassium dihydrogen phosphate buffer (pH 2.9) with 0.1% triethylamine and (B) acetonitrile. The mixture was pumped at a starting gradient of 92% A and 8% B and was adjusted to 75% A and 25% B over 40 min, followed by shift to 85% A and 15% B over 8 min, and back to initial conditions over 6 min. The flow rate was 1.1 ml/min. The fluorescence detector was set at an excitation of 202 and an emission of 296 with the gain at 10×. The column was at ambient temperature.

Tramadol and M1 were extracted from plasma samples using a liquid extraction with a mixture of ethyl acetate and hexane. Briefly, previously frozen plasma samples were thawed and vortexed and 350 μl were transferred to a clean test tube containing 100 μl of internal standard (50 μg/ml butorphanol). Seventy microliters of 29.7% ammonium hydroxide were added followed by 1.5 ml of ethyl acetate : hexane (40 : 60). Tubes were placed on a tube rocker for 15 min followed by

Table 1. Estimated pharmacokinetic parameters (mean, standard error %, lower [L] 95% and upper [U] 95% confidence intervals) after oral administration (in a fish) of a single dose of tramadol (2 mg/kg) to California sea lions (*Zalophus californianus*) ($n = 15$).

Parameter[a]	Estimate	SE %	L 95% CI	U 95% CI
V/F (L/kg)	4.3	17.70	2.4	6.2
K_{01} (1/hr)	3.2	32.97	0.6	5.8
K_{10} (1/hr)	0.32	20.94	0.16	0.49
T_{lag} (hr)	0.72	3.09	0.67	0.78
AUC (hr × ng/ml)	1429	8.13	1145	1714
$t_{1/2}$ (hr)	2.14	20.92	1.04	3.23
CL/F (L/hr per kg)	1.40	8.14	1.12	1.68
T_{max} (hr)	1.52	8.55	1.20	1.84
C_{max} (ng/ml)	357.8	10.44	266.4	449.2

[a] V/F = apparent volume of distribution after nonintravenous administration; K_{01} = rate at which the drug enters the central compartment; K_{10} = rate at which the drug leaves the central compartment; T_{lag} = lag time; AUC = area under the plasma concentration time curve; $t_{1/2}$ = plasma half-life; CL/F = apparent total clearance of drug after nonintravenous administration; T_{max} = time to maximum concentration; C_{max} = maximum concentration.

centrifugation for 20 min at 1,700 *g*. The organic layer was removed into a clean tube and evaporated to dryness under a steady stream of nitrogen gas. Samples were reconstituted in 350 µl of mobile phase and 25 µl were injected into the HPLC system.

Standard curves for plasma analysis were prepared by spiking untreated sea lion plasma with tramadol and M1, which produced a linear concentration range of 5–5000 ng/ml. Spiked standards were treated exactly as plasma samples. Average recoveries were 84 and 93% for tramadol and M1, respectively. Intra-assay variability ranged from 1.1 to 1.2% for M1 and 2.1 to 2.7% for tramadol. Interassay variability ranged from 0.9 to 8.4% and 4.4 to 8.5% for M1 and tramadol, respectively. The lower limit of quantification was 5 ng/ml.

Pharmacokinetic analysis

Average plasma concentration data for parent tramadol were analyzed using a compartmental approach as implemented with Phoenix software (v. 6.3.0.395, Certara USA, Inc., St. Louis, Missouri 63101, USA). 95% confidence intervals for the predicted curve were obtained with the NLIN procedure from SAS 9.4 software (SAS Institute Inc., Cary, North Carolina 27513, USA). The best fitting model was used to simulate concentration-time profiles for different dosing scenarios and target concentrations, and a dosage regimen was proposed based on these. A tramadol concentration of 100 ng/ml was used for the simulation as a threshold for therapeutic efficacy. While the validity of this value as a reference would need to be confirmed with studies designed for this purpose, it was selected based on published information in humans.[9]

RESULTS

Pharmacokinetic study

Pharmacokinetic parameters for the parent tramadol are shown in Table 1. An open monocompartmental model with first order absorption and lag time best fitted the data. The projected average plasma concentrations of parent tramadol at the 2 mg/kg oral dose when placed in fish are shown in Figure 1. As shown, the intestinal absorption of the drug lagged for about 30–45 min after administration. Predicted maximum plasma concentration

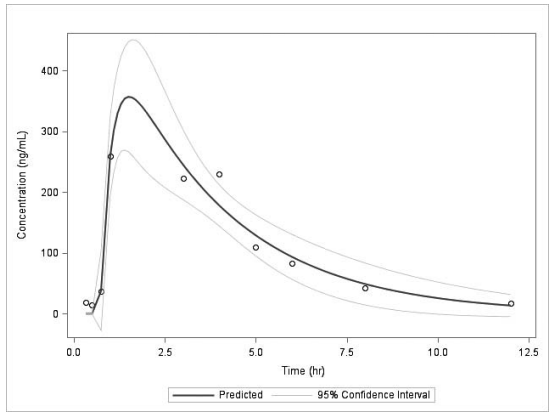

Figure 1. Projected average plasma concentration (ng/ml) versus time (hr) profile after a single, orally administered (placed in a fish) dose (2 mg/kg) of tramadol in California sea lions (*Zalophus californianus*) ($n = 15$).

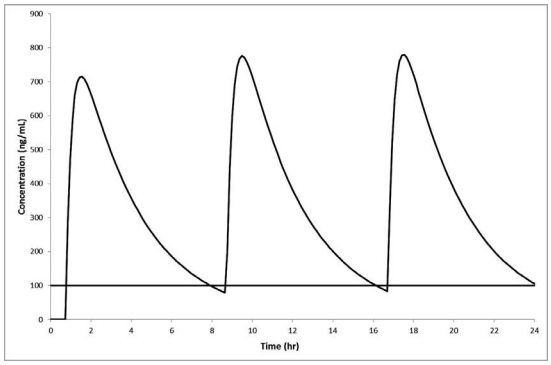

Figure 2. Projected average plasma concentration (ng/ml) versus time (hr) profile after a single, orally administered (placed in a fish) dose (4 mg/kg) of tramadol every 8 hr in California sea lions (*Zalophus californianus*).

approximated 358 ng/ml at 2.14 hr and remained above 100 ng/ml for approximately 6 hr. The volume of distribution relative to the bioavailability was V/F = 4.3 L/kg, the AUC was 1,429.11 hr × ng/ml, and the half-life was short at 2.14 hr. The M1 metabolite was not detectable in 21 of 24 plasma samples, was below the level of quantification of 5 ng/ml in one sample, and was detectable at 11 and 17 ng/ml in two of the samples. None of the animals showed any adverse reactions to oral tramadol.

Pharmacokinetic simulations

Using a best fitting model, further dosing scenarios were explored assuming a 100 ng/ml efficacy threshold. Based on these simulations, a dose of 4 mg/kg q8 hr or q12 hr may be appropriate, on average, for therapeutic purposes. (Fig. 2) The adequacy of this dose should be further explored in efficacy studies.

DISCUSSION

The results obtained in this study emphasize the importance of establishing species-specific pharmacokinetic parameters for tramadol. Pharmacokinetic studies in a range of other species have shown variations in tramadol metabolism not only between species but also within species.[9,20] When comparing pharmacokinetic parameters of parent tramadol obtained in this study to those in the plasma of goats receiving the same 2 mg/kg oral dose, the goats had a similar half-life (2.67 ± 0.54 hr) but a higher maximum plasma concentration (542.9 ± 219.5 ng/ml).[5] Horses given a higher 10 mg/kg oral dose also had a similar half-life (2.14 ± 0.50 hr) but a lower maximum plasma concentration (238 ± 41 ng/ml).[26] Studies in rabbits, dogs, and bald eagles (*Haliaeetus leucocephalus*) show great variation in the maximum plasma concentration when each is given an oral dose of 11 mg/kg. The results from these studies demonstrated a maximum concentration (C_{max}) of 135.3 ± 89.1 ng/ml in rabbits, 1,402.75 ± 695.52 ng/ml in dogs, and 2,156.7 ± 681.4 ng/ml in bald eagles.[10,11,21,22] The plasma concentrations determined to provide adequate analgesic in humans range from 298 ± 171 ng/ml to 590 ± 410 ng/ml for parent tramadol, with 100 ng/ml reported as the minimal level for mild to moderate pain.[5,8,9,20,21,26]

The levels of the M1 metabolite detected in plasma have also been found to be quite variable both interspecies and intraspecies. In humans, the metabolism of tramadol via hepatic demethylation to produce the M1 metabolite is catalyzed by the cytochrome P-450 isoenzyme CYP2D6.[1,16] Studies in humans have found individual variation in the ability to metabolize tramadol to the M1 metabolite, which has been attributed to genetic variability of the CYP2D6 gene.[3,11] The minimally effective analgesic plasma concentration range for M1 in humans is reported as 39.6 ± 29.5 ng/ml to 84 ± 34 ng/ml.[3,5,8,20,21,26] However, some patients considered to be poor metabolizers of tramadol, and which did not reach these levels, still were shown to have analgesic effects from the drug, which suggests that antinociceptive effects may be independent of opioid receptor activity in these individuals.[11,25]

Variations in the metabolism of tramadol to produce the M1 metabolite have also been demonstrated in other species. The results from studies in dogs have demonstrated that M1 was either detected in negligible amounts, present at very low levels, or found in variable amounts in individual dogs.[6,14,27] A study done in greyhounds established the antinociceptive effects with oral tramadol despite M1 levels not exceeding 13.8 ng/ml in any dog, with the greatest analgesic effects achieved at 5 to 6 hrs post drug administration when M1 was less than 1 ng/ml in most of the dogs.[11] Horses have been shown to reach M1 plasma levels of 86.8 ± 17.7 ng/ml following a 10 mg/kg oral dose of tramadol, but M1 was not detected in the plasma samples of any of the horses following intravenous administration of the drug.[26] In contrast, a study in goats demonstrated that M1 was detectable in plasma samples following intravenous tramadol administration but not with oral administration.[5] In avian

species, there were marked differences in M1 metabolism found when comparing studies in peafowl (*Pavo cristatus*) to those in bald eagles.[2]

Overall, the results obtained in this study indicate that tramadol administered orally inside a fish to California sea lions demonstrates low to negligible plasma levels during the first 30–45 min, reaches the target concentration of 100 ng/ml in less than 1 hr, and has an approximate half-life of 2 hr. In this study, tramadol was administered by placing the drug into a herring, which is the preferred method of oral drug administration at TMMC for sea lions able to consume fish on their own. Whether concurrent feeding of fish alters tramadol pharmacokinetics is not known. The cause of the lag-time seen during this study may be in part due to gastric emptying, which may have been prolonged in association with digestion of the fish meal. In a pharmacokinetic study of tramadol in African penguins (*Spheniscus demersus*), an oral dose of 10 mg/kg was also administered by placing the drug into a fish. The results of this study reported tramadol was detected in the plasma of the majority of birds within 15 min of administration and in all of the birds within 30 min. The study also demonstrated that M1 levels were detected within 45 min in all of the birds and remained above the human therapeutic concentrations at 36 hr.[9] Comparison of interspecies pharmacokinetic parameters, however, is often difficult due to differences in physiologic factors and may be affected by the use of fish for drug delivery in this study.

Although the study's small sample size does not allow for statistical comparison, there were no apparent effects of gender or age on tramadol pharmacokinetics. At TMMC, no intoxications have been noted in patients treated with tramadol. As this is the first pharmacokinetic study of an oral pain medication in California sea lions, to the authors' knowledge, further research is needed to determine the potential influences of sex and age on pharmacokinetic parameters as well as any potential for adverse events caused by tramadol.

If a valid goal is to maintain concentrations of parent tramadol above the minimum therapeutic limit of 100 ng/ml, then based on these results the 2 mg/kg oral dose placed in fish would need to be administered every 6–8 hr. Based on simulation of different dosing scenarios also using the 100 ng/ml threshold, a dose of 4 mg/kg every 8 or 12 hr on average may represent an adequate compromise. It is not clear what significance the M1 metabolite being undetectable in the majority of animals following the 2 mg/kg dose of tramadol has in this species. Pharmacodynamic studies are needed to determine if tramadol provides adequate analgesia using the remaining pathways in California sea lions at this or other doses. Large multiple-dose and long-term studies should be conducted to further elucidate tramadol pharmacokinetics, including the effects of sex, age, and concurrent fish consumption, and to adequately determine the safety of tramadol in California sea lions.

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