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Pharmacokinetics of Oral and Subcutaneous Carprofen in Common Marmosets (*Callithrix jacchus*)

Ekaterina Perminov, DVM,[1,*,†] Trinka W Adamson, MS, DVM, DACLAM,[1] and Kuo Fen Lee, PhD[2]

The common marmoset (*Callithrix jacchus*) is an increasingly popular animal model, and while carprofen is a frequently used NSAID in this species, there are no published studies evaluating dosage needs to achieve appropriate analgesia. The aim of this study was to determine the pharmacokinetics of low-dose (2 mg/kg) and high-dose (4 mg/kg) carprofen following oral and subcutaneous routes of administration in marmosets. Three (2 females, 1 male) adult (3.1 ± 1.6 y old [mean ± SD]) common marmosets were used for this study. Blood was collected at 0, 1, 2, 4, 6, 12, and 24 h after administration. The plasma concentrations of carprofen were determined using HPLC and pharmacokinetic parameters. The 4 mg/kg carprofen yielded a significantly higher plasma concentration than did 2 mg/kg carprofen. However, our data show that neither administration route, nor dose, result in plasma concentrations at or above the desired therapeutic threshold. The poor pharmacokinetic properties suggest that these doses of carprofen are not adequate and that either higher doses should be considered or carprofen should not be used as the NSAID of choice in the common marmoset.

Abbreviation and Acronyms: AUC 0-inf, AUC to infinity; COX, cyclooxygenase; T_{last}, time of last measurable concentration

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Introduction

The common marmoset (*Callithrix jacchus*) is a New World primate that has reemerged as a research model with its use rising at an extraordinary rate.[1,52] Publications describing work with marmosets as the animal model have risen from approximately 1,800 in 2000 to 5,300 in 2023. Their small size, short gestation time, ease of breeding in captivity, and low zoonotic risk in comparison to Old World primates make them an attractive laboratory animal model.[39] Resurgence in the use of this species is driven by studies in gene-editing technology, neuroscience, aging, and behavior.[28,45,48] Due to the generation of stable transgenic lines in the common marmoset, the species has been touted as the next 'biomedical supermodel.'[7,54]

According to the *Guide for the Care and Use of Laboratory Animals*, proper use of analgesics in research animals is both an ethical and a scientific imperative.[24] Multimodal analgesia refers to the combination of multiple analgesic drug classes to target different points along the pain pathway for synergistic effects. Doing so optimizes analgesia while reducing the required doses of individual drugs and decreasing the risk of undesirable effects.[15,17,30] Nonsteroidal anti-inflammatory drugs (NSAIDs) are often paired with an opioid analgesic to cover different pain pathway mechanisms, particularly during more invasive procedures.[5,9,15] Carprofen, a commonly used NSAID, inhibits cyclooxygenase (COX) enzymes at the cell membrane that are responsible for prostaglandin synthesis. Carprofen mediates its analgesic, antiinflammatory, and antipyretic effects through selective COX-2 inhibition, while inhibition of COX-1 likely accounts for most of its potential negative side-effects.[10,30] However, carprofen is considered a 'newer generation' NSAID, blocking inflammatory events while preserving some beneficial COX-1 activity.[2] Carprofen is a commonly used NSAID in nonhuman primates.[49] It is also recommended for the treatment of musculoskeletal pain, trauma, and pain associated with mastitis, respiratory disease, and osteoarthritis in canines, cattle, and horses.[50,51]

As mentioned, NSAIDs are often paired with opioids for multimodal analgesia. In the marmoset the most commonly used opioid analgesic is buprenorphine, which comes in 2 forms. One is standard short-acting buprenorphine HCl, while the second is a sustained/extended-release formulation, buprenorphine ER. Pharmacokinetic data for both formulations have been published for common marmosets; however, there are no current pharmacokinetic data for NSAIDs for marmosets. Lack of such studies limits appropriate multimodal analgesia application in this species. Carprofen pharmacokinetic data have been described and published for many common laboratory animals, including mice,[27] rats,[53] dogs,[8,42,55] cats,[57] sheep,[11,36,60] swine,[21] horses,[32,43] rabbits,[23] calves,[13] Japanese quail,[58] and rainbow trout.[59] Carprofen dosing recommendations for the common marmoset are extrapolated from other species or are based on anecdotal evidence, and current suggested dosages range from 2 to 4 mg/kg administered either subcutaneously or intramuscularly.[6,16,22,33]

To effectively and appropriately use carprofen as an analgesic for the common marmoset, determinination of its pharmacokinetic profile is essential. In addition, it is important to assess pharmacokinetics of various dosing routes, including oral, which may affect absorption and plasma clearance. This is particularly important in a nondomesticated species, such as the marmoset, where stress may be associated with the handling

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[1]Animal Resources Department, Salk Institute for Biological Studies, La Jolla, California; and [2]Peptide Biology Laboratories, Salk Institute for Biological Studies, La Jolla, California
**Corresponding author: Email: epermino@uci.edu*
[†]Current affiliation: University Laboratory Animal Resources, University of California, Irvine, Irvine, California.

required during subcutaneous and intramuscular injections. Marmosets are particularly sensitive to stress, and therefore frequent handling, for repeat injections as an example, may increase the amount of stress they experience.[4,25] Therefore, evaluation of orally administered drugs, when possible, is an important consideration when determining a drug choice in this species. The oral route is convenient and may not require restraint if animals voluntarily consume the medication.

The aim of this study was to investigate and compare the pharmacokinetics of oral and subcutaneous administration routes of carprofen at low (2 mg/kg) and high (4 mg/kg) doses in the common marmoset. We hypothesized that the subcutaneous formulations would sustain half-maximal concentrations longer than oral formulations, and that both doses would reach the therapeutic threshold.

Materials and Methods

Animals. Three adult marmosets (3.1 ± 1.6 y old [mean ± SD]) were used to complete this study (one female originally from Texas Biomedical Research Institute, and one female and one male born at the Salk Institute for Biologic Studies). They were part of a marmoset breeding colony housed at the Salk Institute for Biologic Studies, an AAALAC-accredited facility. Historically, the females had been treated for *Giardia* (testing negative posttreatment with tinidazole) and received once a month Estrumate (cloprostenol) injections for birth control. The male had experienced a distal tail amputation and had undergone sperm collection in the past. Pathogens such as *Giardia* and *Clostridium difficile* cycle through the colony and are treated when causing clinical disease.

The study was performed in accordance with the National Research Council's *Guide for the Care and Use of Laboratory Animals*, the Animal Welfare Act as Amended. 2008. 7 USC §2131–2156, and United States Public Health Service policy.[3,24,47] All procedures performed were approved by the Salk Institutional Animal Care and Use Committee. All marmosets in this study were socially housed as either male/female or male/male pairs. They were deemed healthy based on routine physical examination (including body condition scoring, bloodwork [CBC and full chemistry panel], an intradermal tuberculosis test, and fecal culture and analysis for ova and parasites) performed by a veterinarian semiannually prior to enrollment. The marmosets were maintained on a 12-h light/12-h dark cycle, temperature range of 80 ± 2 °F (23.3 ± 1.0 °C), with a relative humidity range of 30% to 70%. Enclosures (2 marmoset Britz and 1 in-house custom-built enclosure) contained perches, nest boxes, hammocks, and hanging toys. The diet consisted of commercial biscuits soaked lightly in orange-flavored Tang (Kraft Heinz, Chicago, IL) fed in the morning (LabDiet New World Primate Diet 5040; Land O'Lakes, Inc, Richmond, IN). The evening meal consisted of a soft canned diet (ZuPreem, Premium Nutritional Products, Mission, KS) supplemented with fresh food items including fruits or vegetables and a protein source (egg, cottage cheese, garbanzo beans) once daily. Enrichment included acacia gum, foraging boxes, and mealworms. Animals were provided reverse osmosis water ad libitum from water bottles. Staff observed animals at least twice daily and individual health records were maintained.

Acclimation. To decrease the potential stress associated with study procedures, animals were acclimated to transport, capture, handling, and venipuncture restraint position prior to initiating the study. Animals were acclimated to capture into the transport cage with positive reinforcement using a high food item of choice (marshmallow, banana chips, pudding, marshmallow fluff). Once the animal voluntarily entered the transport cage they were acclimated to restraint and appropriate venipuncture handling position, also with positive reinforcement. Acclimation was performed in daily 30- to 40-min sessions for a minimum of 5 continuous days prior to study initiation. Acclimation was repeated prior to use in the next analgesic group.

Study design and drug administration. A crossover design was used such that each of the 3 animals received all 4 treatment groups. The 3 animals were randomized into each group, that is, low dose orally, high dose orally, low dose subcutaneously, and high dose subcutaneously, prior to study initiation. Once an experimental group was determined, prior to administration of the drug, each marmoset was weighed to calculate an accurate dose. Animals received the drug while conscious and restrained by a handler. For subcutaneous administration of carprofen (Rimadyl 50 mg/mL; Zoetis, Kalamazoo, MI), animals received either a single 2 or 4 mg/kg SC injection between shoulder blades. The interscapular region was shaved prior to subcutaneous drug administration for visual monitoring of potential injection site reactions.

Oral carprofen formulation was composed of flavor tablets (Rimadyl 50 mg/mL; Zoetis, Kalamazoo, MI) reconstituted in deionized water. Animals received either a high dose (4 mg/kg) or low dose (2 mg/kg) of reconstituted carprofen by mouth at a given time point. They then had their blood drawn for pharmacokinetic analysis as described below. All animals had a minimum 10-d washout period before use in the next randomized, analgesic treatment. No animals were used in the same analgesic group twice. The low and high doses chosen were based on suggested published doses,[22,37] and what has historically been used at our institution.

Sample collection. Blood samples (approximately 0.2 to 0.4 mL) were collected using either via saphenous or femoral venipuncture into heparinized BD Microtainer blood collection tubes (BD, Inc.; Franklin Lakes, NJ). Blood samples were collected from all analgesic groups immediately prior to analgesic administration at a baseline time point (0 h) and then subsequently at time points 1, 2, 4, 6, 12, and 24 h after drug administration. For saphenous blood collection, one person manually restrained the animal and provided positive reinforcement treats such as marshmallow, banana chips, pudding, or marshmallow fluff, while a second person performed phlebotomy from the saphenous vein. All blood samples were collected via the saphenous vein, except the 12-h time point, to rest the saphenous vein after the first 5 blood collections. During the 12-h time point, animals were anesthetized with isoflurane (3% to 4% in 1.5 L/min oxygen for induction, 1% to 3% for maintenance) via face mask, and blood collection was performed via femoral venipuncture. Anesthesia was used to safely immobilize the animal for the procedure. Animals were provided atropine (Hikma Pharmaceuticals, Berkeley, NJ) at 0.02 mg/kg IM to control tracheobronchial secretions. Blood collection volume amounts (0.2 to 0.8 mL) were replenished as a lactated Ringer solution fluid (Covetrus; Portland, ME), which was administered subcutaneously at every other blood draw. The marmosets were returned to a transfer cage between sample collection time points. Transfer cage location was maintained within the same light cycle, temperature range, and relative humidity as animal holding rooms. Animals were allowed to return to their home cage after the 24-h blood collection. Blood samples were quickly processed after collection by centrifugation (10,000 × g) for 10 min, and plasma was separated and stored in cryogenic tubes at –80 °C until analysis.

Table 1. Weights in grams of study animals at baseline and at completion of each 24-h study period

	First dose		Second dose		Third dose		Fourth dose	
	Before	After	Before	After	Before	After	Before	After
Young male	500	486	499	480	510	490	516	493
Young female	456	436	445	424	438	426	437	428
Old female	479	451	466	460	472	461	517	502

Observations. All marmosets were observed at each blood collection time point within the transfer cage during the study and for up to 24 h after within their holding cage. Marmosets were evaluated for general wellbeing and for potential adverse effects after drug administration. Temperature, pulse, respiration, mentation, urination, defecation, and food and water intake were noted daily, and any abnormalities were recorded.

Sample analysis. Carprofen plasma concentrations were measured by liquid chromatography–MS. Deuterated carprofen was added as an internal standard to 5- to 20-µL aliquots of plasma samples. Samples were extracted with 400 µL of an acetonitrile/methanol mixture (1:1, v/v). The samples were vortexed and centrifuged (20,000 × *g*, 10 min at 4 °C). The supernatants were transferred to another tube and evaporated, and the extracts were reconstituted in 50 µL of water/methanol (1:1, v/v) and transferred to an autosampler vial for liquid chromatography–MS analysis.

Liquid chromatography–MS analysis was performed on a Dionex UltiMate 3000 LC system (Thermo Fisher Scientific; Waltham, MA) coupled to a TSQ Quantiva mass spectrometer (Thermo Fisher Scientific; Waltham, MA). A Kinetex (Phenomenex; Torrance, CA) C18 column (2.6 µm, 150 × 2.1 mm) was used. Solvent A consisted of 0.1% formic acid in water, and solvent B was 0.1% formic acid in methanol. The gradient was increased linearly from 0% to 100% in solvent B in 12 min, held at 100% in solvent B from 12 to 18 min, returned to 0% in solvent B, and held at 0% in solvent B until 25 min. MS analyses were performed using electrospray ionization in a positive mode, with spay voltages of 3.5 kV, ion transfer tube temperature of 325 °C, and vaporizer temperature of 275 °C. Sheath, auxiliary, and sweep gases were 15, 10, and 1, respectively. Multiple reaction monitoring was performed for carprofen (274.1 > 167.1, 274.1 > 193.1, 274.1 > 228.1) and d3-carprofen (277.1 > 170.1, 274.1 > 196.1, 274.1 > 231.1) as an internal standard. Skyline[34] was used to extract and quantitate peak areas. Carprofen concentrations were determined from the peak area ratios of the analyte relative to its internal standard and compared with the calibration curve generated from the analysis of human plasma spiked with known concentrations of the analyte and its internal standard.

Data analysis. Statistic and noncompartmental pharmacokinetic analysis were performed by using the PK package[61] in R (version 4.2.0), with code available on GitHub. C_{max} and T_{max} were determined based on direct observation of concentration–time data. The observed AUC (AUC 0–time of last measurable concentration [T_{last}]) was calculated using the log-linear trapezoidal rule, and the AUC to infinity (AUC 0-inf) was calculated assuming exponential decay of the last 3 time points. The T_{last} was decided at the time of the study design as 24 h and was the same for each marmoset. The last measurable concentration was directly observed at T_{last}. The other parameters are functions of the AUC and dosage. Mean resident time was calculated by dividing area under the first moment curve by AUC 0-inf. Terminal half-life was calculated by multiplying mean resident time by the natural log. Total clearance was calculated by dividing the dose by AUC 0-inf. The volume of the distribution at steady state was calculated by multiplying the clearance by the mean residence time. Bioavailability issues are unlikely to have influenced clearance and volume of distribution, and this was an assumption made during statistical analysis in our study, because carprofen is almost 100% bioavailable after oral dosing and after subcutaneous administration in other species.[11,12,53,55,59] Oral and subcutaneous carprofen exposure has been deemed to be bioequivalent, with comparable absorption under steady-state conditions, despite differing peak plasma concentrations.[55]

Results

Observations. All marmosets remained healthy throughout the entirety of the study. Adverse effects were temporary and resolved without intervention. Adverse effects included hyporexia, skin lesions, and weight loss. Up to 10% weight loss from initial body weight was noted during the 24-h study period. Weight loss was attributed to stress of handling and anesthesia. Table 1 provides baseline body weights and weights at completion of each 24-h cycle after carprofen administration (following the 24-h blood collection). Weights returned to baseline by the next study time point in all but the young female, which was noted to have mild progressive weight loss of 6.14% from baseline weight during the study period.

Skin lesions (Figure 1) developed after subcutaneous injections of carprofen. Lesions were seen in 4 of the 6 subcutaneously injected sites within 12 h. They occurred in the young male with the 2 and 4 mg/kg dose, in the young female with the 4 mg/kg dose, and in the old female with the 2 mg/kg dose. Lesions were less than 0.5 cm in circumference, superficial, and mild, with surrounding erythema with no obvious swelling, pain, or discharge present. All skin lesions resolved within 10 d.

Pharmacokinetic analysis. Plasma concentrations of 2 carprofen doses and 2 administration routes were determined in 3 marmosets over a 24-h period (Figure 2). Baseline blood samples at time 0 demonstrate no detectable carprofen in the plasma after washout periods.

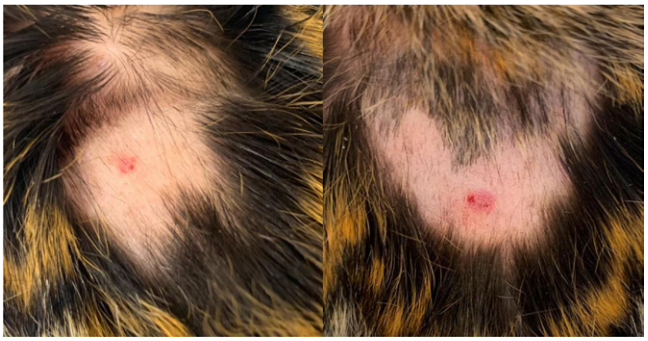

Figure 1. Representative skin lesions with mild superficial localized erythema after subcutaneous carprofen injections. Four out of 6 instances when marmosets received subcutaneous carprofen injections, minor lesions developed within 12 h. No swelling was noted at the injection sites, and animals were not observed to experience pain. Lesions were attributed to nonspecific dermatitis.

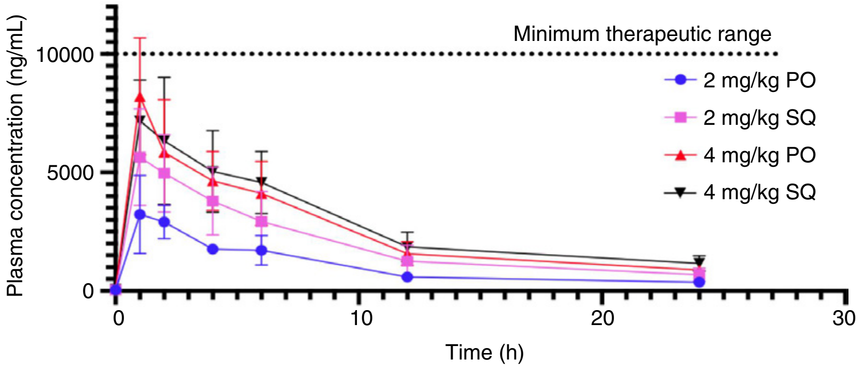

Figure 2. Plasma concentration–time curve showing mean plasma concentration (error bar, SD) after 2 mg/kg PO (*n* = 3), 2 mg/kg SC (*n* = 3), 4 mg/kg PO (*n* = 3), or 4 mg/kg SC (*n* = 3) administration in marmosets. The dashed line indicates minimal therapeutic range to reach based on the canine determined therapeutic plasma concentration (10,000 ng/mL = 10 µg/mL).[46]

Plasma concentrations after administration of 2 mg/kg carprofen showed a rapid and robust increase (Figure 2). The peak plasma concentration when administered orally was calculated to be 3170.37 ± 1.51 ng/mL and achieved at 1.26 ± 1.49 h after administration. For the subcutaneous route, peak plasma concentration of 5,414.43 ± 1.43 ng/mL was calculated and achieved at 1 ± 0 h after administration. The plasma concentrations declined by 24 h after administration to 359.83 ± 1.27 ng/mL for the oral route and 649.39 ± 1.51 ng/mL for the subcutaneous route. After 4 mg/kg carprofen administration either orally or subcutaneously, there was a similar rapid and robust increase in the plasma concentration (Figure 2). For the oral route, a peak plasma concentration of 8,005.74 ± 1.33 ng/mL was calculated and achieved at 1 ± 0 h after administration. For the subcutaneous route, the peak plasma concentration was calculated to be 7,011.61 ± 1.27 ng/mL and was achieved at 1.26 ± 1.49 h after administration. After 24 h the plasma concentrations declined to 903.33 ± 1.21 ng/mL for the oral route and to 1,106.49 ± 1.34 ng/mL for the subcutaneous route. T_{max} at 24 h did not differ significantly in regard to route or dose. Bioavailability for 2 mg/kg PO compared with subcutaneous dosing was calculated to be 55.28%. Comparatively, the 4 mg/kg PO compared with the subcutaneous dosing was calculated to be 94.44%. The bioavailability difference between 2 mg/kg compared with 4 mg/kg is likely to be due to nonlinear pharmacokinetics, and the bioavailability of carprofen appears to be dose-dependent.

Table 2 provides the complete pharmacokinetic parameters for both low-dose (2 mg/kg) and high-dose (4 mg/kg) carprofen administered orally and subcutaneously.

Discussion

To the best of our knowledge, this is the first study assessing pharmacokinetics of carprofen in the common marmoset. Studies have shown a wide range of plasma concentrations after various carprofen dosages administered via a variety of routes in other species. The determined therapeutic plasma concentrations based in the dog range from 10 to 17 µg/mL.[46] The therapeutic levels of carprofen in other species based on in vitro assays evaluating inhibition of cyclooxygenase indicate a range of 20 to 24 µg/mL.[31] The therapeutic plasma concentration of carprofen in humans with rheumatoid arthritis is 10 µg/mL.[14] Our results show that neither route used at either dose provided the appropriate increase to plasma concentration of carprofen above the intended therapeutic threshold. The 4 mg/kg carprofen administered orally was calculated to have a mean C_{max} of 8,005.74 ng/mL, estimated to 8 µg/mL, which was below the determined therapeutic plasma concentration of 10 to 17 µg/mL. To achieve the targeted therapeutic plasma concentration of carprofen in marmosets, a dose higher than 4 mg/kg would be required. Based on comparative calculations of the C_{max} from 4 mg/kg carprofen administered orally, the dosage to achieve an intended therapeutic plasma concentration of 10 to 24 µg/mL would need to be at a range of 5 to 12 mg/kg. An alternative would be to use allometric dose extrapolation. Using the FDA allometric scaling based on the marmoset allometric exponent with an original 4 mg/kg carprofen dosage, an equivalent 7.2 mg/kg allometric dosage of carprofen should be used.[19,56] The $t_{1/2}$ results for all doses and routes indicate the necessity of twice daily dosing, which is consistent with the current prescribing information.[40,50,51]

Table 2. Pharmacokinetic parameters after administration of carprofen to common marmosets (*n* = 3)

	2 mg/kg PO	2 mg/kg SC	4 mg/kg PO	4 mg/kg SC
C_{max} (ng/mL)	3,170.37 ± 1.51	5,414.43 ± 1.43	8,005.74 ± 1.33	7,011.61 ± 1.27
T_{max} (h)	1.26 ± 1.49	1 ± 0	1 ± 0	1.26 ± 1.49
$t_{1/2}$ (h)	8.28 ± 1.13	8.42 ± 1.03	8.5 ± 1.08	9.93 ± 1.14
AUC 0-last (ng·h/mL)	24,921.65 ± 1.3	45,165.58 ± 1.51	61,624.81 ± 1.29	65,250.51 ± 1.34
AUC 0-inf (ng·h/mL)	29,650.77 ± 1.27	53,793.58 ± 1.5	73,537.23 ± 1.25	82,511.33 ± 1.33
C_{last} (ng/mL)	359.83 ± 1.27	649.39 ± 1.51	903.33 ± 1.21	1,106.49 ± 1.34
T_{last} (h)	24	24	24	24
Volume steady state (L/kg)	0.81 ± 1.37	0.45 ± 1.5	0.67 ± 1.36	0.69 ± 1.36
Clearance (L/h·kg)	0.07 ± 1.27	0.04 ± 1.5	0.05 ± 1.25	0.05 ± 1.33
MRT (h)	11.94 ± 1.13	12.15 ± 1.03	12.26 ± 1.08	14.33 ± 1.14

Geometric means ± SD are shown. Animals in 2 mg/kg SC and 4 mg/kg PO groups reached a similar T_{max} without variability. C_{last}, last measurable concentration; MRT, mean resident time.

Table 3. Serum chemistry values from 2 adult colony marmosets during semiannual physical examination before carprofen and 3 to 5 d after administration of 2 mg/kg SC carprofen

Parameter	Female: Before carprofen	Female: 5 d after carprofen	Male: Before carprofen	Male: 3 d after carprofen
Total protein (g/dL)	6.6	4.2	6.1	5.1
Albumin (g/dL)	5.7	2.9	3.8	3.0
Globulin (g/dL)	0.9	1.3	2.3	2.1
AST (IU/L)	169	15,603*	23	813*
ALT (IU/L)	11	703*	4	13
ALP (IU/L)	51	384	81	390
GGT (IU/L)	9	18*	5	6
BUN (mg/dL)	15	57*	17	108*
Creatinine (mg/dL)	0.3	2.7*	0.4	3.6*
Phosphorus (mg/dL)	7.5	7.0	5.5	16.5*
Glucose (mg/dL)	10*	234	46*	237
Calcium (mg/dL)	10.6	8.5	10.1	8.2
Sodium (mEq/L)	161	143	155	138
Potassium (mEq/L)	4.0	2.4	4.0	9.0*
Chloride (mEq/L)	102	90	105	84

Values marked with an asterisk deviate from published reference intervals, indicating marked abnormalities in hepatic parameters, severe azotemia, and notable electrolyte disturbances.[29]

Carprofen can be associated with potential negative side effects even at recommended dosing ranges. These include mucosal injury to the gastrointestinal tract and nephropathy.[20] Carprofen dosed from 1.57 to 3.2 mg/kg (mean of 2.34 mg/kg) administered every 12 h has been associated with development of hepatocellular toxicosis in a retrospective study of 21 dogs.[35] Interestingly, carprofen dosed subcutaneously to CD-1 mice at the published dosage of 5 to 10 mg/kg was shown to reach therapeutic plasma levels;[40,41] however, neither the 5 mg/kg dose nor the 10 mg/kg dose reached analgesic efficacy. In another study using CD-1 mice, carprofen administered subcutaneously at a dosage of 20 mg/kg in CD-1 mice was shown to be relatively safe with no renal, hepatic, or gastrointestinal side effects.[26] The current recommended sheep dose based on pharmacokinetic parameters is 8 mg/kg administered daily.[36,50] The only side effect reported in sheep after intramuscular carprofen administration is an increase of plasma creatine kinase attributed to muscle damage.[11] Therefore, carprofen dosing, efficacy, and safety appear to be variable.

This study did not assess the pharmacokinetics of carprofen doses higher than 4 mg/kg due to experience with carprofen toxicity in 2 adult (4.3 ± 0.3 y old) colony marmosets (1 male, 1 female, from the same dam and sire) after administration during routine care. Carprofen (Rimadyl 50 mg/mL; Zoetis, Kalamazoo, MI) at a 2 mg/kg dosage was administered subcutaneously as an analgesic during microchip placement while animals were anesthetized with isoflurane. Prior to carprofen administration, blood was collected from both animals to assess serum chemistry as a component of semiannual physical examination with no major abnormalities noted (Table 3). The male marmoset declined 3 d after carprofen administration while the female declined 5 d after such administration. Clinically, both animals presented with lethargy, hypothermia, bradycardia, weight loss, and dehydration. In addition, the male common marmoset experienced hematochezia. Blood was collected from both animals prior to euthanasia to assess chemistry values. Serum chemistry parameters of both animals revealed marked elevations in hepatic values, severe azotemia, hypoproteinemia, and electrolyte disturbances (Table 3).

Necropsy revealed moderate hemorrhagic enteritis, moderate hepatic lipidosis, mild early spontaneous glomerulonephritis for the male, and severe hemorrhagic and necrotizing gastritis, enteritis, and colitis, with associated gastric ulceration for the female. Based on history, presentation, serum chemistry changes, and histologic interpretation of tissues by an outside diagnostic laboratory, the diagnosis of carprofen toxicity was made for both animals. The incident prompted careful review of dosing records and administration practices, but no evidence of overdose or deviation from standard dosing protocols were identified. These isolated toxicities highlight the potential for individual hypersensitivities or other unidentified contributing factors. A potential contributing factor may have been dehydration and/or isoflurane anesthesia at the time of carprofen injection. Isoflurane does not directly interact with carprofen; however, it may indirectly influence the distribution and clearance of carprofen through its effects on cardiac output, vasodilation, and blood pressure reduction.[50] No evidence of toxicity was noted in animals enrolled in the current study.

Cutaneous lesions were observed in 4 of the 6 subcutaneous injection sites in this study and with similar frequency in the low- and high-dose subcutaneous groups (Figure 1). No significant cutaneous effect differences were noted between first and second subcutaneous injections. The skin lesions were mild, superficial, transient, resolved quickly, and were attributed to nonspecific dermatitis. Carprofen animal safety information describes similar dermatologic changes, including slight redness and rash, along with swelling and warmth at the injection site. Rare injection site reactions including necrosis, abscess and seroma formation, necrotizing panniculitis, and ventral ecchymosis are listed as rare situational side effects on the prescribing information.[51] Therefore, continued monitoring of injection sites is recommended.

One criticism of this current study is the small number of animals used for each treatment group. The toxicity in the 2 marmosets described above occurred toward the end of our study window, and given the low carprofen plasma levels found in the first 3 animals, it was determined that working with more animals would unlikely lead to different conclusions than what was being observed for the 2 and 4 mg/kg dosages. Repeating the study with higher carprofen dosages, while giving us the

opportunity to collect more data, did not seem prudent considering the toxicity as described above.

A recommendation against the use of carprofen in marmosets cannot be made solely on pharmacokinetic data, which is another limitation of this study. Further research is necessary to evaluate the analgesic efficacy in this species, although such experiments are inherently challenging and complex.[18] Plasma concentrations are often used to estimate drug exposure and guide dosing; however, they may not accurately reflect drug distribution to target sites.[44] Changes in protein binding and vascular permeability due to inflammation can significantly affect tissue drug concentrations.[38] Therefore, studies that integrate tissue pharmacokinetics with plasma data are warranted to provide a more comprehensive understanding. NSAIDs are commonly used in a multimodal analgesia approach for marmosets; however, data on synergistic or additive effects remain scarce. Further investigation would be beneficial to assess the benefits and risks of multimodal protocols. Ultimately, this study found that carprofen, when administered at 2 or 4 mg/kg, failed to achieve adequate therapeutic levels in common marmosets.

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Conflict of Interest

The authors have no conflicts of interest to declare.

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