Pharmacokinetics of carprofen in lactating dogs after intravenous treatment

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Abstract

Analgesia is an important component of veterinary care with nonsteroidal antiinflammatory drugs, particularly carprofen (Rimadyl®) commonly used for dogs. Pharmacokinetic studies of carprofen in lactating dogs have not been examined fully, questioning the use and safety of carprofen. The extent to which lactation changes the pharmacokinetics is unknown, nor is the extent of transfer into milk of lactating dogs and their neonates or pups. We hypothesized that after a single dose of intravenous (4.4 mg/kg) to dogs, concentrations in milk are low and not high enough to produce harmful exposure in pups. We tested our hypothesis in healthy, adult lactating dogs (n = 4) using pharmacokinetic methods and nonchiral and chiral specific assays measuring total, R-, and S-enantiomer carprofen concentrations with high performance liquid chromatography in maternal plasma, milk, and neonatal plasma samples. The C_{MAX}, elimination half-life, and clearance for maternal plasma were 9.09 and 7.3 µg/ml (R-, S+), 6.82 and 6.22 hours (R-, S+), and a higher clearance rate of 95.81 ml/hr/kg (R-) and 73.87 ml/hr/kg (S+) than reported. None of the neonatal plasma concentrations at any time point were more than 10% of the total maternal plasma concentrations and milk:plasma ratio was < 1. This study confirmed the approved dose is appropriate for perioperative and chronic pain in lactating dogs and is safe with reduced exposure to nursing pups. This study suggested that the pharmacokinetics of carprofen in lactating dogs may be different from their nonlactating counterparts. However, further pharmacokinetic studies with more dogs are needed to confirm these findings.

Keywords: Lactation, analgesia, carprofen, neonate, pup, pharmacokinetics

Introduction

Carprofen, a preferential COX-2 (PTGS2; cyclooxygenase-2 or prostaglandin-endoperoxide synthase-2 enzyme) inhibitor first evaluated in dogs in 1990,[1] is approved for perioperative and chronic pain management in dogs.[2-5] Carprofen is a racemic nonsteroidal antiinflammatory drug (NSAID) that has antiinflammatory, analgesic, and antipyretic properties. Carprofen consists of 2 enantiomers R(-) and S(+), with the S(+) enantiomer having greater antiinflammatory activity compared to the R(-) enantiomer. Carprofen ordinarily has a good safety profile;[3,6-8] however, it has adverse effects common to all NSAIDs, such as kidney, liver, and gastrointestinal injury.[1,2,6-9] An adverse reaction specific to dogs is an idiosyncratic acute hepatopathy, with an incidence ranging from < 0.05 to 1.6%.[9,10]

Pharmacokinetics for carprofen have been reported in normal, healthy, nonlactating dogs[1] and in canine induced-pain models.[5,9,11-14] Following cesarean surgery, some dogs experienced pain or had preexisting problems (e.g. osteoarthritis)[15] that warranted analgesia. Carprofen has been used anecdotally in dogs undergoing cesarean surgery as a single subcutaneous dose without adverse effects reported in either dogs or neonates.[16-18] The FDA approved label for carprofen states, "The safe use of Rimadyl® in animals less than 6 weeks of age, pregnant dogs, dogs used for breeding purposes, or in lactating bitches has not been established."[9] This labeling may discourage effective pain treatment and control in dogs having recently whelped or are lactating.

Studies in women report that medications with greater than 85% protein binding produce low concentrations in breast milk.[19-25] Carprofen is 99% protein-bound in canine plasma and is a weak acid (pKa = 4.3);[1] therefore, it is less likely to pass from the plasma to milk and is not a candidate for ion trapping via the pH-partition theory. It has a low volume of distribution making it less likely to diffuse out of the

intravascular space into the body tissue, even in inflammatory conditions.[12,13] In a study, dogs that received a 5-day course of carprofen following cesarean surgery were assigned to 3 groups: 'normal' (88), 'mastitis' (4), and 'generalized inflammation' (8).[18] Maternal plasma and milk sample concentrations were compared, and dogs with mastitis had higher concentrations of carprofen in the milk compared to the normal and generalized inflammation groups. Predicted expected neonatal exposure was < 1 (milk:plasma ratio) in all groups.[18] These authors concluded that carprofen was safe to treat lactating dogs; however, no pharmacokinetics analysis was performed in that study to provide further information on the disposition of carprofen in lactating dogs.

Our hypothesis is based on the work performed with other NSAIDs in people and other animals and results from carprofen concentrations in canine milk. Minimal data are available regarding milk concentrations for carprofen in dogs,[17,18] but no pharmacokinetic studies have been reported that provide direct measurements of neonatal plasma concentrations, or to examine if the pharmacokinetics in lactating dogs are different from other dogs. The purpose of our descriptive study was to determine the pharmacokinetic disposition of carprofen in plasma of lactating dogs, measure concentrations in canine milk of treated dogs, and measure concentrations in plasma of nursing pups. We predicted that a single intravenous dose of carprofen in dogs will fail to produce milk concentrations in lactating dogs that exceed the limit of quantification in milk.[18] We also hypothesized that pups nursing from dogs after a single intravenous carprofen treatment will not have detectable concentrations of carprofen in their plasma.

Materials and methods

Exclusion/inclusion criteria

Seven dogs were enrolled in the study and recruited from privately owned kennels with approval via consent form and a factual sheet on carprofen provided from the manufacturer. The ages of the dogs were 2.5-6.0 (average 4.34) years and weighing between 8.91 and 27.27 kg (average 21.03 kg). Litters ranged from 16-39 days of age (average 28 days); pups weighed more than 300 grams and were being fed exclusively maternal milk. Dogs and litters consisted of 2 breeds: American foxhound (n = 6) and Cavalier King Charles spaniel (n = 1). Enrollment criteria were: a. healthy based on physical examination; b. uneventful whelping or delivery; c. at least 14 days postpartum; and d. had no exposure to NSAIDs in the 30 days prior to enrollment. Physical examinations of dogs and visual assessment of pups were performed at the start of the study prior to enrollment to determine whether they were healthy enough for the study. Daily weight gains were not tracked during the study, but pups were weighed and monitored by the breeders prior to the start of the study to ensure they were over 300 grams. Dogs were housed in their normal kennels with their owners/breeders and samples were taken on-site without transportation of any animal; dogs met enrollment criteria. Three dogs were excluded shortly after samples were started due to: a case of mastitis present at milk collection, difficulty in venous access for sampling, and inability to collect milk from a third dog. Four out of 7 litters successfully provided complete sets (maternal plasma, milk, and pup plasma). All 4 litters sampled ranged from 16-24 days old, with the majority of the pup diet consisting of milk, but food was available for dogs at all times. The study and experimental design were reviewed and approved by the Institutional Animal Care and Use Committee at North Carolina State University (Protocol 21-382).

Experimental design

Each dog received a single intravenous carprofen at the FDA approved subcutaneous/oral dose of 4.4 mg/kg between 14-39 days postpartum after blank plasma and milk samples were collected from each dog (time = 0). Although the intravenous route of treatment is not included on the approved label for dogs in the US, we chose an intravenous route (extra-label use) because: a. there were no reported adverse effects observed in dogs from past experimental studies[1,8,13] and in the UK; b. intravenous dose ensures 100% bioavailability that is important for pharmacokinetic calculations of clearance and volume of distribution; and c. our aim was to calculate transfer of drug from plasma to milk, irrespective of treatment route. A single milk and plasma sample prior to treatment was collected and measured to serve as a control for each female, and then a series of milk and simultaneous plasma samples were collected and measured following treatment at predetermined time points: 0, 0.5, 1, 1.5, 2, 8, 12, 24, 36, 48, and 72 hours.[13]

Repeated blood sampling can be stressful to young pups; therefore, a sparse sampling strategy was used for pups in the same litters that were at least 14-days-old. This involved taking a maximum volume of 0.17 ml. This volume would not exceed a 10% blood loss over the entire collection period for a pup weighing 300 grams. For each time point, blood from at least 3 pups within the same litter was pooled that provided sufficient sample for that time point for analysis. The pharmacokinetic analysis performed used a naïve averaged data approach on a rotational basis to minimize the handling and venipuncture of each pup.[25] Milk was collected via manual expression into sterile 2-ml cryovials, with volumes measuring 20-1,000 μl, and stored at −80°C until analysis. Blood was collected via venipuncture into sterile 2-ml heparinized tubes at a maximum of 4 attempts per dog or pup. The samples were immediately placed on ice following collection, centrifuged (20,000 x g 10 minutes) within 30-90 minutes after acquisition, the plasma was pipetted off and placed into sterile 2-ml cryovials, and plasma stored at −80°C until analysis.

High performance liquid chromatography

High performance liquid chromatography (HPLC; or high pressure liquid chromatography) was performed on all samples using validated methods developed in our laboratory.[13,26] Maternal plasma, milk, and pooled neonatal plasma samples were analyzed via HPLC to determine concentrations of carprofen in plasma and milk using methods modified from previous studies.[10,13,26] The HPLC system consisted of a 4-solvent delivery system, an autosampler, and fluorescent detection with an emission wavelength set at 310 nm and detection wavelength set at 375 nm (Agilent 1100 Series: Agilent Technologies, Wilmington, DE, USA).[13] The chromatogram was integrated with proprietary software (OpenLab CDS, Agilent Technologies, Wilmington, DE, USA). Two columns were used for analysis, depending on the sample analyzed. All maternal plasma was analyzed with a chiral column (Ultron ES-OVM) in a column oven of 40°C that produced separation of the R- and S-enantiomers with the R-enantiomer eluting from the column first. Because of the smaller volume available for analysis, all milk and neonatal samples were analyzed with a nonchiral column (Zorbax Eclipse XDB-C18 column, Agilent Technologies). The mobile phase for the chiral

column was isocratic at 85% 0.02 M potassium phosphate in water and 15% acetonitrile at a flow rate of 1 ml/minute. The mobile phase for the nonchiral column was set to 55% 0.02 M potassium phosphate in water and 45% acetonitrile at a flow rate of 1 ml/minute. The methods used were validated in our laboratory for canine plasma. A fresh calibration curve (described below) and quality control (QC) samples were analyzed with each batch of samples to ensure that the assay met our acceptance criteria.

Calibration standards

A stock solution of carprofen was prepared using a racemic reference standard from the US Pharmacopeial Convention and dissolved in methanol to 1,000 µg/ml This was stored at refrigeration temperature in a closed container and used to fortify blank the matrix for standard calibration samples for all 3 sampling groups.[26-28] The calibration curve for maternal plasma consisted of 6 standards ranging from 0.1-50 µg/ml and 5 standards ranging from 0.05-1 µg/ml for both milk and pup plasma of the racemic mixture. Since specific enantiomer concentrations were being measured for maternal plasma, enantiomer specific calibration curves were based on R- and S+ concentrations of 0.05-25 µg/ml as R- and S+ are in a 50:50 ratio in the racemic mixture. Calibration curves were accepted if the linear coefficient of determination (r^2) was ≥ 0.99. The limit of detection (LOD) was 0.01 µg/ml and the limit of quantification (LOQ) was 0.03 µg/ml, based on the lowest point on a linear calibration curve that met our acceptance criteria.

Sample preparation

Preparation for all plasma, milk, calibration, and blank samples were identical aside from volume of sample and 4% phosphoric acid due to sample volume available. Samples were thawed at room temperature the day of preparation, and 500 µl was diluted in a 1:1 ratio with an equivalent volume of 4% phosphoric acid in water and then centrifuged for 5 minutes at 20,124 x g. The diluted sample was loaded into labelled preconditioned solid-phase extraction cartridges (Oasis Prime HLB 1 ml; Waters Corporation, Milford, MA, USA), and washed with a 5% methanol solution. Carprofen was eluted into clean tubes with 1 ml of a 90% acetonitrile and 10% methanol solution. The eluates were evaporated at 40°C and 20 psi for 25 minutes. The dry residue was reconstituted with 250 µl of the mobile phase. The samples were vortexed briefly and 200 µl was transferred to HPLC injection vials, and 20 µl was injected onto the column.

Pharmacokinetics

The concentrations measured for maternal and neonatal plasma were averaged at each timepoint and plotted on nonlogarithmic and logarithmic scale against time to initially select the best model for pharmacokinetic analysis. Pharmacokinetic analysis was performed with commercial pharmacokinetic software (Phoenix® WinNonlin™ and NLME®, Certara, St. Louis MO). Pharmacokinetic models were fitted to the data to determine the best fit.[25,26,29-31] Two compartment modeling is ideal for intravenous treatment as the central compartment, or first compartment, is the intravenous space and the second compartment is the extravascular volume. This type of modeling considers the movement between the 2 compartments. One dog was excluded from the pharmacokinetic analysis (dog 4) because of inadequate samples collected during the first 24 hours, resulting in only a few concentrations in the elimination phase of the curve. Because there were only 4 litter samples and 1 milk sample being above the LOQ, there were not enough data to perform a pharmacokinetic analysis on dog milk or pup plasma.

Results

All maternal plasma carprofen concentrations were below the LOD by 24 hours. Postcarprofen treatment (0-72 h) concentrations ranged from 0 µg/ml to 6.11 µg/ml for the R(-) enantiomer and 0 µg/ml to 10.85 µg/ml for the S(+) enantiomer (Figure 1).
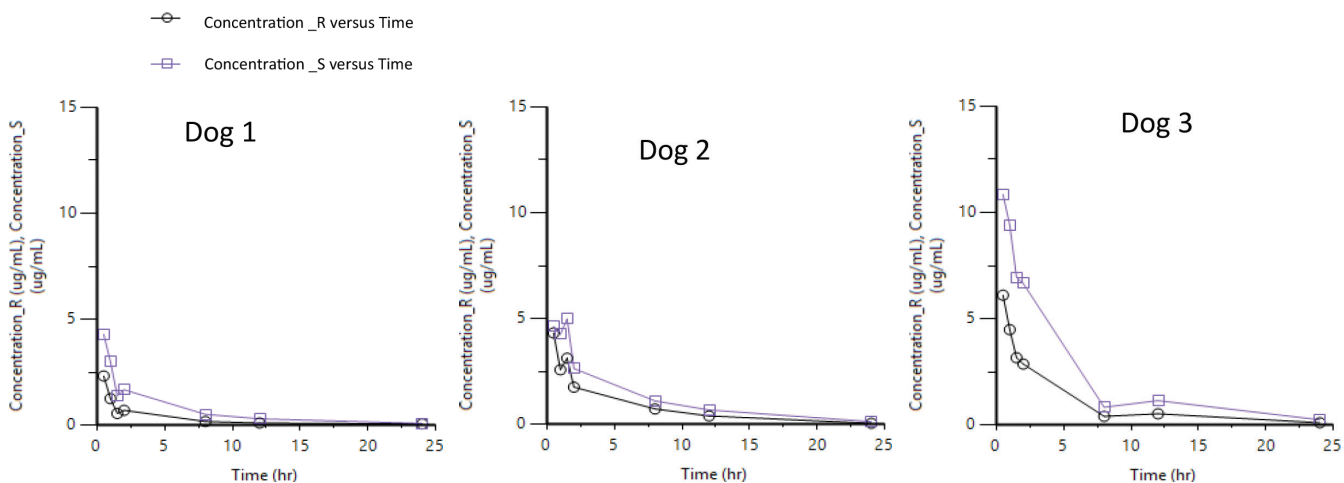
Figure 1. Pharmacokinetics of carprofen in dog's plasma after a single intravenous (4.4 mg/kg) carprofen treatment between days 16 and 24 postpartum.

A 2-compartment pharmacokinetic modeling system on a nonlogarithmic scale was used to graph the R-enantiomer concentrations of carprofen (black circles) compared to the S-enantiomer concentrations of carprofen (purple squares) against time for plasma samples from 3 dogs (between days 16 and 24 postpartum).
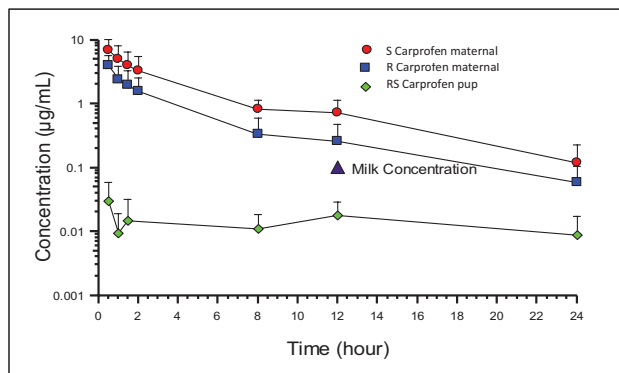
Figure 2. Carprofen concentrations in maternal plasma, maternal milk, and pup's plasma after a single intravenous carprofen (4.4 mg/kg) treatment to lactating dogs between days 16 and 24 postpartum.

Maternal carprofen concentrations in plasma averaged for each timepoint out to 24-hours on a logarithmic scale broken into R- and S-enantiomers concentrations, total carprofen concentrations in neonatal plasma, and the single milk sample above LOQ. Litters' concentrations were also averaged together for each timepoint. S-enantiomer concentrations in red circles, R-enantiomer concentrations in blue squares, pup plasma concentrations in green diamonds, and milk as a black triangle.

Most pup samples were below the LOD for most time points with only 17 out of 35 samples being above the LOD. Out of these 17 samples; however, only 4 were above

the LOQ. The majority were in litter 1 (0.5, 1.5, and 12 hours postmaternal treatment) and the last was at the first time point for litter 2 (0.5 hour postmaternal treatment). Milk carprofen concentrations were above the LOQ and LOD only at a single timepoint (12 hours) in dog 1. None of the neonatal plasma concentrations at any time point were more than 10% of the total maternal plasma concentrations (Figure 2).

Twenty-one concentration-time samples (time 0.5-24 hours) from 3 dogs (16, 22, and 24 days postpartum for dogs 1, 2, and 3 respectively) were included into pharmacokinetic modeling for both R- and S-enantiomer of carprofen. Our study identified multiple differences in the pharmacokinetic parameter estimates compared to studies. We observed a higher clearance rate compared to studies in healthy and pain-induced dogs.[8,11,13,31,32] We also observed a higher volume of distribution in our dogs compared to studies – the volume of distribution for the central compartment was 2-3 times higher than reported. Enantiomer-specific primary parameter estimates for total (protein bound and unbound carprofen) are provided (Table).

Discussion

Because of uncertainty regarding the safety of carprofen to nursing pups after carprofen treatment to lactating dogs, we studied the disposition of carprofen in dogs' plasma and milk, and in nursing pups' plasma. This information was needed because carprofen is one of the most prescribed analgesics for perioperative use, mastitis, metritis, and osteoarthritis in dogs that may be lactating. Although we were unable to analyze

Table. Pharmacokinetic parameters for carprofen R(-) and S(+) enantiomers of maternal plasma in a 2-compartment model in 3 dogs after an intravenous dose of 4.4 mg/kg

Parameter	Units	R(-) enantiomer		S(+) enantiomer	
		Median	Range	Median	Range
A	µg/ml	7.7	(3.3-374.5)	6.0	(2.9-12.8)
α	1/hour	1.2	(0.8-11.3)	0.7	(0.4-1.3)
B	µg/ml	1.4	(0.3-3.1)	3.0	(1.3-3.1)
β	1/hour	0.1	(0.1-0.2)	0.1	(0.1-0.1)
αHL	hour	0.6	(0.1-0.8)	1.0	(0.5-1.6)
βHL	hour	6.8	(4.0-12.6)	6.2	(5.2-6.8)
AUC	hour*µg/ml	23.0	(7.5-51.5)	29.8	(15.9-47.9)
AUMC	hour*µg/ml	106.5	(86.4-147.2)	192.3	(105.4-311.7)
CL	ml/hr/kg	95.8	(43.0-292.9)	73.9	(46.0-138.7)
C_{MAX}	µg/ml	9.1	(3.6-377.6)	7.3	(5.8-15.7)
k10	1/hour	0.5	(0.4-7.4)	0.3	(0.2-0.5)
k12	1/hour	0.6	(0.3-3.8)	0.2	(0.1-0.7)
k21	1/hour	0.2	(0.1-0.3)	0.3	(0.2-0.3)
MRT	hour	6.4	(2.1-11.5)	6.5	(6.5-6.7)
V1	ml/kg	242.1	(5.8-611.8)	301.3	(139.8-378.0)

A and B: Y-axis intercepts for the phases of distribution and elimination; α and β: fractional elimination rate constants; k10, k12, k21: elimination and compartmental rate constants; MRT: mean residence time; k10 HL: elimination half-life; α-HL: distribution half-life; β-HL: terminal half-life; AUC, area-under-the-curve; V1, volume of the central compartment; Cl, total plasma clearance. Estimates are based on a 2-compartment model.

pharmacokinetics for all testing groups, these results supported our hypothesis that carprofen is safe to treat lactating dogs because there is low observed milk concentrations and negligible risk to pups.

Concentrations of carprofen in the maternal plasma of lactating dogs were below the LOD by 24 hours that was expected for the dose (for 24 hours at 4.4 mg/kg) given and comparable to studies in healthy (intravenous, subcutaneous, and oral routes) and pain-induced (subcutaneous and oral routes) dogs.[1,14,15,33] Plasma concentrations in lactating dogs were different than published for nonlactating dogs in both enantiomers.[1,10,14] The major differences observed between our results and earlier studies were higher clearance rates and volumes of distribution and lower AUCs. The maximum concentration, movement between both compartments and elimination rates were similar. Reasons for these differences are undetermined, but it may be due to higher plasma volume during lactation and underlying physiological inflammation associated with uterine involution.[34] Changes in plasma volume have been documented to increase by 23% in lactation for sheep[35] and 45% in pregnant women.[32] In women, plasma concentrations of some drugs have been reported to change during pregnancy and albumin concentrations undergo a steady decline to ∼ 70-80% of normal values at the time of delivery.[32] When albumin concentrations decreased, there is a significant increase in the AUC of unbound drugs that can increase the pharmacological effect. In addition, estradiol and progesterone increased hepatic metabolism via xenobiotics as well as CY-P450 enzyme activity.[32] Evidence suggested that an increased clearance and decreased concentrations of some drugs may be the results of these physiologic changes.[32]

Only a few studies described physiological changes that occur during canine pregnancy with limited studies during lactation. Most reported hematologic and serum biochemical differences in pregnant versus nonpregnant dogs having anemia, thrombocytosis, leukocytosis, hypoalbuminemia, hypoproteinemia, and changes in cholesterol amongst other findings in late pregnancy.[31] An increase in plasma volume during pregnancy in dogs has been noted,[36] but an exact percentage increase has not been calculated to the authors' knowledge. With this increase in plasma volume, increased renal blood flow and glomerular filtration rate have been observed.[37] We theorized that changes in women and other species in pregnancy and lactation are similar in dogs and can explain our observations.

The low detection of carprofen in the milk in our study was consistent with our predictions of low transfer from mother to milk, due to the partition theory as well as carprofen's high percentage of protein binding in the plasma (> 98%),[1] most of which is bound to albumin. The single milk sample with concentrations above the LOQ occurred at 12 hours after treatment in dog 1 coinciding with litter 1 which measured above the LOQ at that timepoint as well. This mother was the heaviest dog (23.3 kg) in our study; she was also the oldest and one of the better milk producers in the kennel having raised several litters (exact number unknown). She was of similar lactation stage as the other dogs at 22 days into milk with a similar litter size of 7 pups. Without further investigation, we cannot speculate if dog size and/or milk production alone contributes to the extent of disposition of carprofen in milk and pup exposure. We were not able to accurately measure total milk ingestion in pups nor daily milk production in the dog during our study.

There were only 4 timepoints in pups from 2 litters with carprofen concentrations above the LOQ. This agreed with our hypothesis that carprofen distribution to milk is low and exposure to nursing pups is too low to produce measurable pup plasma concentrations. Most of the samples with concentrations above the LOQ were from litter 1. Overall, pup plasma concentrations were less than 10% of maternal concentrations, consistent with our hypothesis and observation from human infant exposure.[19-22]

Despite our small sample size, the low and unquantifiable concentrations of carprofen in milk were similar to the low concentrations reported[18] after a 5-day oral treatment of carprofen; that study had slightly higher concentrations of carprofen in the milk compared to our study that may be attributed to the difference in the stage of lactation during the sampling period. At the beginning of lactation, the volume of milk produced is smaller and colostrum is excreted. Therefore, larger-sized proteins will pass more readily through the mammary gland barrier in the first 24-hours as well as a higher concentration due to the smaller volume of milk produced at this time. In our study, dogs were at 2-4 weeks of lactation in which the mammary epithelial tight junctions are more restrictive and higher milk volume produces a dilution factor. Other differences are the sampling and analyzing technique. We chose an intensive sampling model after a single dose of carprofen for both maternal plasma and milk along with plasma from nursing puppies, whereas once daily sampling of maternal milk over a 5-day course of oral carprofen (2 mg/kg twice per day) was chosen.[18] By only collecting milk samples once a day, changes in milk concentrations (foremilk versus hindmilk and interglandular variations) may be missed that has been described in human lactation studies.[38] Milk samples were analyzed by liquid chromatography[18] as in the current study. The main difference in analyzing techniques involved the maternal and neonatal plasma that were calculated via concentration-time course simulation models.[18] versus in real time in the current study.

Limitations

A major limitation of our study was the small sample size that resulted in high inter-individual variation among dogs. Clearly, a larger study with more dogs is needed to confirm our results. Aside from the small sample size, another limitation was the single carprofen treatment before steady-state conditions were met. Because we used dogs recruited from client-owned kennels there were limitations for the number of doses and samples from dogs and pups. We were restricted in the volume of whole blood that could be collected for an individual pup during the sampling period for that litter. Although we used an intravenous treatment that is not included in approved US label, this was the preferred route for calculating pharmacokinetic parameters such as clearance and volume of distribution. Furthermore, intravenous route studies in dogs had no adverse effects in dogs.[1,13] However, there was not enough data from the milk and pups to calculate pharmacokinetic parameters from these samples.

Despite these limitations, this is the first study to our knowledge that has measured carprofen in neonatal plasma and canine milk at multiple timepoints after maternal treatment, an advance over studies in dogs.

In conclusion, carprofen treatment to lactating dogs has low transference to milk and a low risk of neonatal exposure in midlactation with an intact mammary epithelium barrier.

Carprofen disposition observed in lactating dogs in our study had a higher clearance rate and volume of distribution that may be attributed to the unique physiology in this group compared to nonlactating dogs. Our study supported our hypothesis that carprofen is safe for lactating dogs as there is low transfer into their milk and to their pups, which is similar to reports.[16,18]

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Conflicts of Interest

Amber Nebel-Karp: No conflicts of interest to disclose.

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