

Pharmacokinetics of orally administered DL- α -lipoic acid in dogs

Steven C. Zicker, DVM, PhD; Albert Avila, PhD; Dinesh K. Joshi, PhD; Kathy L. Gross, PhD

Objective—To determine the pharmacokinetics of DL- α -lipoic acid in dogs when administered at 3 dosages via 3 methods of delivery.

Animals—27 clinically normal Beagles.

Procedures—In a 3 × 3 factorial Latin square design, 3 dosages (2.5, 12.5, and 25 mg/kg) of DL- α -lipoic acid were administered orally in a capsule form and provided without a meal, in a capsule form and provided with a meal, and as an ingredient included in an extruded dog food. Food was withheld for 12 hours prior to DL- α -lipoic acid administration. Blood samples were collected before (0 minutes) and at 15, 30, 45, 60, and 120 minutes after administration. Plasma concentrations of DL- α -lipoic acid were determined via high-performance liquid chromatography. A generalized linear models procedure was used to evaluate the effects of method of delivery and dosage. Noncompartmental analysis was used to determine pharmacokinetic parameters of DL- α -lipoic acid. Nonparametric tests were used to detect significant differences between pharmacokinetic parameters among treatment groups.

Results—A significant effect of dosage was observed regardless of delivery method. Method of delivery also significantly affected plasma concentrations of DL- α -lipoic acid, with extruded foods resulting in lowest concentration for each dosage administered. Maximum plasma concentration was significantly affected by method of delivery at each dosage administered. Other significant changes in pharmacokinetic parameters were variable and dependent on dosage and method of delivery.

Conclusions and Clinical Relevance—Values for pharmacokinetic parameters of orally administered DL- α -lipoic acid may differ significantly when there are changes in dosage, method of administration, and fed status. (*Am J Vet Res* 2010;71:1377–1383)

R- α -Lipoic acid is a coenzyme for several intramitochondrial α -keto-dehydrogenases (eg, pyruvate dehydrogenase) and is used as an antioxidant or mitochondrial nutrient in many animal species.^{1,2} Activity of pyruvate dehydrogenase is decreased in older animals; however, this may be mitigated by providing increased concentrations of α -lipoic acid in the diet.³ Furthermore, it has been suggested⁴ that α -lipoic acid should be considered as a conditionally essential mitochondrial nutrient.

α -Lipoic acid may be obtained by the ingestion of foods that contain lipoyllysine or by de novo biosynthesis within mammalian cells.³ α -Lipoic acid occurs in nature as an amino acid-bound R- α enantiomer, lipoyllysine, which is believed to undergo minimal cleavage before gastrointestinal absorption.⁵ Cysteine is the source of sulfur and octanoate is the intermediate precursor for the 8-carbon fatty acid used during de novo

ABBREVIATIONS

AUC _{0-last}	Area under the time-concentration curve from time 0 to the time of the last quantifiable concentration
C _{last}	Last quantifiable concentration
C _{max}	Maximum serum concentration
HPLC	High-performance liquid chromatography
λ_z	Elimination rate constant
T _{max}	Time of maximum concentration
T _{1/2}	Elimination half-life

biosynthesis of α -lipoic acid. A protein-bound complex is usually the product of de novo α -lipoic acid biosynthesis.^{1,2,6} Only minor amounts of unbound α -lipoic acid derived from ingested food or de novo biosynthesis are found in the noncellular mammalian blood matrix.⁷⁻⁹ Most of the free form of α -lipoic acid is partitioned into intracellular compartments, with the highest concentrations detected in liver, heart, and kidney.⁷⁻⁹

When radiolabeled DL- α -lipoic acid is administered orally to dogs, > 60% of the radiolabel undergoes urinary excretion within 24 hours after administration, and overall, 80% is renally excreted within 72 hours.¹⁰ However, most of the radiolabeled compounds recov-

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From Hill's Pet Nutrition Incorporated, Pet Nutrition Center, 1035 NE 43rd St, Topeka, KS 66601.

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Address correspondence to Dr. Zicker (Steven_Zicker@hillspet.com).

ered from the urine of treated dogs are metabolites of DL- α -lipoic acid. It is presumed that when DL- α -lipoic acid is administered orally to dogs, the compound will undergo mitochondrial β -oxidation in the liver, which results in a high first-pass metabolism effect.¹⁰ When compared with other animal species, mitochondrial β -oxidation of DL- α -lipoic acid in the liver of dogs results in many similar as well as unique metabolites.¹⁰ However, metabolic patterns of polar metabolites in urine such as methyl sulfoxides, which are more prevalent in dogs, compared with mice, rats, and humans,¹⁰ varied from species to species.

In addition to metabolism differences among animal species, method of delivery may influence the absorption of DL- α -lipoic acid from the gastrointestinal tract. The T_{\max} of DL- α -lipoic acid when administered orally in humans at dosages between 50 and 600 mg is between 0.5 and 1 hour and displays a dose-proportional relationship. The bioavailability of enantiomers of α -lipoic acid when administered orally in humans is decreased in solid formulations, compared with the bioavailability of α -lipoic acid in aqueous formulations.¹¹ Additionally, concurrent food intake (fed status) significantly reduced bioavailability of α -lipoic acid when administered orally in humans.^{11,12} Investigators have reported¹³ differences in bioavailability between the 2 enantiomers of α -lipoic acid in humans.

Bioavailability of DL- α -lipoic acid when manufactured as an ingredient in a nutritionally complete dog food has not been directly assessed. However, dogs fed extruded foods that included DL- α -lipoic acid as an ingredient resulted in an increase of the reduced glutathione-to-oxidized glutathione ratio in WBCs, which is consistent with improved antioxidant status.¹⁴ During the processing of nutrients in commercial pet foods, adducts of compounds may occur and impact bioavailability as seen with Maillard products and lysine. The purpose of the study reported here was to evaluate the effect of method of delivery and dosage on pharmacokinetic parameters of DL- α -lipoic acid in dogs when administered in a capsule form and provided without a meal, in a capsule form and provided with a meal, or included as an ingredient in a nutritionally complete extruded dog food.

Materials and Methods

Animals—Twenty-seven clinically normal adult Beagles with ages ranging from 3.7 to 13.5 years (mean \pm SD, 7.8 ± 2.8 years) were used for this study. The dogs included 13 neutered males, 1 sexually intact male, 12 spayed females, and 1 sexually intact female. A physical examination was performed on each dog, and all dogs were vaccinated against canine distemper virus, adenovirus, parvovirus, bordetellosis, and rabies. Because factors such as disease and inflammation could have confounded the results of the study, a CBC and serum biochemical analysis were performed to verify the health status of each dog.

Dogs were selected for their ability to consume an entire daily ration in ≤ 30 minutes after the ration was offered. Dogs were housed in pairs and fed once daily. Dogs received behavioral enrichment through dog-

dog interactions, daily interactions and play time with caretakers, and access to toys. The study protocol was reviewed and approved by the Hill's Pet Nutrition Institutional Animal Care and Use Committee.

Food formulation—A nutritionally complete extruded canine maintenance food^a was fed to all dogs on all of the nontreatment days in an amount sufficient to achieve the daily caloric intake requirement of each animal based on historical records for each animal. Three variations of the base extruded food were specially formulated with DL- α -lipoic acid^b to provide a target dosage of 2.5, 12.5, or 25 mg of DL- α -lipoic acid/kg when administered orally on treatment days. Proximate analysis of the extruded dog foods included measurements for moisture content, protein, fat, ash, calcium, and phosphorous at a commercial laboratory.^c

Capsules containing DL- α -lipoic acid—Quantities of a powder containing DL- α -lipoic acid^b were weighed specifically for each dog to provide target dosage of 2.5, 12.5, or 25 mg of DL- α -lipoic acid/kg. The weighed quantities were then inserted into No. 1 lock-ring gelatin capsules.^d Capsules of DL- α -lipoic acid were administered orally by hand by a caretaker.

Study design—Treatments were administered by following a 3×3 factorial Latin square study design. Dogs were randomly assigned to 1 of 9 treatment groups. Treatment groups comprised 3 dogs for each of 3 treatments. Treatment groups included the oral administration of 2.5, 12.5, and 25 mg of α -lipoic acid/kg dosages that were in a capsule form and provided without a meal; 2.5, 12.5, and 25 mg of DL- α -lipoic acid/kg dosages that were in a capsule form and provided with a meal; and 2.5, 12.5, and 25 mg of DL- α -lipoic acid/kg dosages that were included as an ingredient in a nutritionally complete extruded dog food. All treatments were separated by a minimum washout period of 7 days. Food was withheld from all treatment groups for 12 hours before the start of each phase of the study. Water was supplied ad libitum.

Sample collection—A vacutainer system was used for the collection of 2 mL of blood from a jugular vein immediately before (0 minutes) and 15, 30, 45, 60, and 120 minutes after treatment administration into evacuated tubes with K₂EDTA. Tubes were centrifuged at $1,462 \times g$ for 10 minutes at 4°C to separate plasma from the cellular fraction. One-milliliter aliquots of plasma were then transferred to microcentrifuge tubes and stored at -70°C until quantitation of DL- α -lipoic acid was performed.

Quantitation of DL- α -lipoic acid concentration in food and plasma by use of HPLC—Quantitation of DL- α -lipoic acid concentration in the extruded maintenance and specially formulated extruded dog foods was performed by use of a previously described analytic method.¹⁴ Quantitation of DL- α -lipoic acid concentration in canine plasma was performed by use of a previously described¹⁵ HPLC method with some modifications. Samples were thawed and allowed to equilibrate to 25°C and then mixed. One hundred microliters of each plasma sample was

removed and transferred to a separate 2-mL microcentrifuge tube. Ten microliters of a 100 mg/mL solution of TCEP (tris[2-carboxyethyl]phosphine hydrochloride in water) was added to each sample, and samples then were mixed. Samples were then incubated at 4°C for \geq 30 minutes. One hundred microliters of a sulfosalicylic acid solution (6% sulfosalicylic acid and 1mM EDTA in water) was added to each sample, and samples then were mixed. Samples were incubated for 10 minutes at 25°C. Samples were then centrifuged at $18,700 \times g$ for 11 minutes at 5°C. A 100- μ L aliquot of the supernatant solution from each sample was removed and transferred into a new 2-mL microcentrifuge tube. Two hundred microliters of a borate buffer (2.5M borate, 4mM EDTA [pH, 10.5]) and 100- μ L of an SBD-F solution (1 mg of an ammonium salt of 7-fluorobenzofurazan-4-sulfonic acid/mL added to the borate buffer [pH, 9.5]) were added to each sample. Samples were mixed and then centrifuged at $3,700 \times g$ for 5 minutes at 5°C. Then, samples were incubated in a water bath at 60°C for 1 hour. Samples were removed from the water bath and cooled at 4°C for 20 minutes before being transferred to HPLC autosampler vials.

Chromatography was performed by use of an HPLC system^e equipped with a solvent degasser, quaternary pump, autosampler, column-heater compartment, and fluorescence detector. The α -lipoic acid derivative was analyzed via reversed-phase HPLC at 25°C by use of an HPLC analytic column^f (length, 150×4.6 mm; diameter, 5- μ m) and a guard cartridge.^g Separation of analytes was performed by use of a gradient elution with a flow rate of 1 mL/min. Mobile phase A comprised methanol (proportion, 2%) and 0.1M phosphate buffer (pH, 6; proportion, 98%). Mobile phase B comprised methanol (proportion, 50%) and water (proportion, 50%). A mobile phase gradient was used as follows: 0 minutes, 50% of A and 50% of B; 10 minutes, 100% of B; 15 minutes, 100% of B; 16 minutes, 50% of A and 50% of B; and 21 minutes, 50% of A and 50% of B. A 50- μ L injection of each sample was used in the analysis. The α -lipoic acid derivative was quantified by use of fluorescences at 385 nm (excitation) and 515 nm (emission) and a photomultiplier tube gain of 15. A designation was not made between the concentration of the D and L enantiomers of α -lipoic acid during HPLC analysis. A commercial software program^h was used to control the HPLC analysis and process the data. Analytic method parameters were determined before analysis of the samples from the study. A series of 7 standard dilutions of α -lipoic acid were prepared daily; a linear correlation coefficient of > 0.99 was determined for all days in which HPLC analysis was performed. Precision of the HPLC analytic method was determined by the calculation of a relative SD for the results of 5 plasma samples analyzed 3 times each on 3 separate days. The range of the relative SD was between 3.5% and 8.7%. Accuracy was determined by the results of the overall recovery of known amounts of α -lipoic acid that were used to spike plasma samples 3 times at 2 concentrations on 3 separate days. Overall recovery of the 2 concentrations was 94.6% and 97.3%. The limit of detection and limit of quantitation of the method were 5 and 15 ng/mL, respectively. Stability of DL- α -lipoic acid in the frozen plasma samples was not determined.

Pharmacokinetic analysis—Noncompartmental analysis was used to determine pharmacokinetic parameters (ie, $AUC_{0\text{--}last}$, C_{last} , C_{max} , λ_z , T_{max} , and $T_{1/2}$) from observed data by use of a commercial software program.ⁱ Results of HPLC analysis for dogs that did not consume $\geq 80\%$ of the targeted dose were not included in the pharmacokinetic analysis. Results of HPLC analysis that were < 30.1 ng/mL were included in the noncompartmental model as 0 ng/mL because the reported result was less than the level of quantitation for the HPLC method.

Statistical analysis—Effects of method of delivery and dosage were evaluated by use of the generalized linear models procedure.^j A value of $P < 0.05$ was considered significant for all analyses. Parameters derived by noncompartmental analysis were also analyzed with a commercial software program.^j Because of the skewness of data, a Kruskal-Wallis test for nonparametric data was used to evaluate the effect of treatment. A 2-tailed value of $P < 0.05$ was considered significant for all analyses.

Results

Physical examinations and analysis of the results of CBCs and serum biochemical analyses did not reveal chronic systemic disease in any of the dogs included in this study. Results of the analysis and concentrations of DL- α -lipoic acid (as fed) in the extruded maintenance dog food and the 3 specially formulated variations of the extruded maintenance dog food were summarized (Table 1). Five dogs (1 dog orally administered the 2.5 mg of DL- α -lipoic acid/kg dosage that was included in an extruded food, 2 dogs orally administered the 12.5 mg of DL- α -lipoic acid/kg dosage that was included in an extruded food, 1 dog orally administered the 25 mg of DL- α -lipoic acid/kg dosage that was in a capsule form and provided with a meal, and 1 dog administered the 25 mg of DL- α -lipoic acid/kg dosage that was included in an extruded food) did not receive $\geq 80\%$ of the target dose. Therefore, results for these 5 dogs were excluded from all subsequent analyses. Included in the analyses were results for the 3 dogs that had consumed DL- α -lipoic acid dosages in excess of the targeted dosages, which was attributable to overeating. Instead of being provided a 2.5 mg of α -lipoic acid dosage that was included in an extruded food, 1 dog was offered and consumed a dosage of 3.4 mg/kg and another dog was offered and consumed a dosage of 4.9 mg/kg. The third dog was offered and consumed a dosage of 15.7 mg/kg instead of a dosage of 12.5 mg/kg that was included in an extruded food. In all 3 instances, the actual dosage of α -lipoic acid consumed was used as the dosage in the noncompartmental pharmacokinetic analyses.

HPLC analysis—In 3 dogs at 1 time point each, plasma concentrations of DL- α -lipoic acid were 3 to 8 times as high as concentrations of other dogs in the study. These increased plasma concentrations were detected 30 minutes after treatment in 2 dogs; this occurred in 1 dog administered dosages of 2.5 and 12.5 mg of DL- α -lipoic acid/kg in a capsule form and provided without a meal and at 15 minutes for the other

Table 1—Results of proximate analysis and concentrations of α -lipoic acid (as fed) in a nutritionally complete extruded dog food^a and 3 variations of the extruded dog food formulated to provide target dosages of 2.5, 12.5, or 25 mg of α -lipoic acid/kg.

Variable	Extruded dog foods			
	Maintenance	2.5 mg of α -lipoic acid/kg	12.5 mg of α -lipoic acid/kg	25 mg of α -lipoic acid/kg
Moisture content (%)	8.2	8.1	8.3	8.5
Protein (%)	23.5	23.9	23.3	23.9
Fat (%)	15.2	14.9	14.6	15.4
Ash (%)	4.9	5.0	4.8	5.1
Calcium (%)	0.74	0.81	0.74	0.81
Phosphorus (%)	0.54	0.58	0.56	0.59
α -Lipoic acid (g/mL)*	15	191	602	1,145

One sample was analyzed for each extruded dog food.
*A designation was not made between the concentrations of the D and L enantiomers of α -lipoic acid during the analysis.

Table 2—Range, mean \pm SD, and median values for pharmacokinetic parameters determined via noncompartmental analysis for DL- α -lipoic acid in plasma samples obtained from Beagles orally administered DL- α -lipoic acid in a capsule form and provided with or without a meal and included as an ingredient in an extruded dog food.

Parameter	2.5 mg of α -lipoic acid/kg			12.5 mg of α -lipoic acid/kg			25 mg of α -lipoic acid/kg		
	Capsule without meal	Capsule with meal	Extruded dog food	Capsule without meal	Capsule with meal	Extruded dog food	Capsule without meal	Capsule with meal	Extruded dog food
C_{max} (ng/mL)									
Range	96–2,389	48–336	38–68	208–6,238	163–2,758	58–249	491–12,845	1,195–12,536	191–3,084
Mean \pm SD	529 \pm 724*	206 \pm 91*	47 \pm 12*	1,696 \pm 1,847*	1,151 \pm 960*	154 \pm 59*	4,037 \pm 4,171*	5,441 \pm 3,777*	628 \pm 994*
Median	263 (9)	44 (9)	43 (5)	1,167 (9)	1,038 (9)	140 (7)	3,478 (9)	4,973 (8)	290 (8)
C_{last} (ng/mL)									
Range	37–303	33–73	37–70	54–268	34–250	58–168	72–523	94–628	104–271
Mean \pm SD	97 \pm 80*	46 \pm 13*	46 \pm 13*	99 \pm 68†	126 \pm 77†	126 \pm 35†	321 \pm 159†	302 \pm 180†	214 \pm 56†
Median	74 (9)	43 (9)	43 (5)	74 (9)	154 (9)	135 (7)	278 (9)	282 (8)	240 (8)
T_{max} (min)									
Range	15–30	15–30	45–120	15–60	15–60	30–120	15–120	15–60	15–120
Mean \pm SD	21.7 \pm 7.9*	23.3 \pm 7.9*	105.0 \pm 33.5*	35.0 \pm 13.0†	30.0 \pm 16†	62.1 \pm 41†	51.7 \pm 31.9†	22.5 \pm 16†	41.3 \pm 35.6†
Median	15 (9)	30 (9)	120 (5)	30 (9)	30 (9)	45 (7)	60 (9)	15 (8)	30 (8)
AUC_{0-last} (min ng/mL)									
Range	2,129–54,668	1,408–9,928	3,649–6,227	13,802–158,684	12,700–73,895	4,642–18,136	27,152–423,428	77,940–296,289	15,634–67,722
Mean \pm SD	12,340 \pm 16,357†	6,071 \pm 2,417†	4,429 \pm 1,053†	63,958 \pm 45,469*	46,409 \pm 22,271*	13,648 \pm 4,397*	160,016 \pm 137,603*	188,569 \pm 96,902*	30,486 \pm 15,948*
Median	7,253 (9)	6,296 (9)	4,138 (5)	61,469 (9)	50,173 (9)	14,398 (7)	120,133 (9)	186,710 (8)	27,212 (8)
λ_z (min ⁻¹)									
Range	0.019–0.069	0.010–0.074	—	0.016–0.035	0.008–0.033	0.0013–0.0037	0.006–0.048	0.015–0.035	0.0003–0.0145
Mean \pm SD	0.044 \pm 0.021†	0.040 \pm 0.027†	—†	0.026 \pm 0.007*	0.019 \pm 0.010*	0.002 \pm 0.001*	0.028 \pm 0.018*	0.025 \pm 0.008*	0.005 \pm 0.005*
Median	0.036 (6)	0.03 (7)	0.0012 (1)	0.026 (8)	0.02 (8)	0.0017 (4)	0.03 (4)	0.025 (7)	0.0035 (6)
$T_{1/2}$ (min)									
Range	10–36.6	9.4–72	—	19.8–42.8	20.8–85.3	187.6–515.3	14.4–125.1	19.7–44.9	47.9–2,336.2
Mean \pm SD	19.4 \pm 9.7†	28.6 \pm 22.6†	—†	29.1 \pm 8.4*	45.1 \pm 22.2*	379.2 \pm 137.6*	46.7 \pm 52.5*	30.2 \pm 10.0*	573.3 \pm 877.8*
Median	19.2 (6)	22.9 (7)	565 (1)	27 (8)	43.9 (8)	407.1 (4)	23.6 (4)	27.2 (7)	234.8 (6)

Values in parentheses are the number of dogs.
*Within a row within a dosage, values differ significantly ($P < 0.05$). †Within a dosage, values were not significantly different.
— = Not determined because only 1 result was reported because of a negative or 0 slope that was obtained via logarithmic transformation.

dog administered a dosage of 25 mg of α -lipoic acid/kg that was included as an ingredient in an extruded dog food. The results for these dosages in these 2 dogs were included in the noncompartmental and statistical analyses. Furthermore, 3 of 8 dogs treated with a 2.5 mg of DL- α -lipoic acid/kg dosage, included as an ingredient in an extruded food, had plasma concentrations below the limit of quantitation for the HPLC method at all time points. A significant effect of dosage on mean DL- α -lipoic acid plasma concentrations over time was detected for all methods of delivery. In addition, an overall significant effect of method of delivery on mean α -lipoic acid plasma concentrations over time was detected for all dosages administered.

Noncompartmental analysis—Range, mean \pm SD, and median values for pharmacokinetic parameters of α -lipoic acid determined via noncompartmental analysis were summarized (Table 2). It is

noteworthy that the terminal slope on the semi-logarithmic plots for all plasma concentrations of DL- α -lipoic acid in dogs fed the extruded foods at all dosages were almost 0 or > 0 . Thus, few subjects were qualified for estimation of λ_z ; therefore, all variables that were calculated by use of λ_z , including $T_{1/2}$ and AUC_{0-last} , may not have accurately defined the true pharmacokinetic profile for DL- α -lipoic acid when included in an extruded dog food.

C_{max} —Dosage had a significant effect on C_{max} regardless of the method of delivery. A significant difference was detected for method of delivery within each dosage group. Dogs orally administered DL- α -lipoic acid at dosages of either 2.5 or 12.5 mg/kg displayed the highest C_{max} when DL- α -lipoic was administered in a capsule form and provided without a meal. The second highest C_{max} from these same 2 dosage groups was detected in dogs administered a capsule form and pro-

vided with a meal. Conversely the highest mean plasma concentration in the group of DL- α -lipoic acid at the dosage of 25 mg/kg was observed when administered the oral capsule form provided with a meal. The second highest mean plasma concentration in this same group was observed in dogs administered a capsule form provided without a meal. A significantly lower mean C_{\max} was detected at all dosage levels when DL- α -lipoic acid was included as an ingredient in an extruded dog food, compared with the mean C_{\max} when DL- α -lipoic acid was orally administered in a capsule form and provided with or without a meal.

C_{last} —A significant effect of route of delivery was only detected for the 2.5 mg of α -lipoic acid/kg dosage; this may have been attributable to the higher C_{\max} of DL- α -lipoic acid achieved when administered in a capsule form after a 12-hour food withdrawal. The C_{last} was not significantly different between methods of delivery when DL- α -lipoic acid was administered at the 2.5 mg/kg dosage in a capsule form and provided with a meal or administered as an ingredient in an extruded dog food. There was no effect of delivery method on C_{last} for the 12.5 or 25 mg/kg dosages.

$AUC_{0-\text{last}}$ —Method of delivery had a significant effect on $AUC_{0-\text{last}}$ for dogs orally administered the 12.5 and 25 mg/kg dosages of DL- α -lipoic acid. A comparison between plasma concentrations when DL- α -lipoic acid was administered orally in a capsule and provided with or without a meal did not reveal a significant difference in plasma concentrations at any dosage and suggested that the significant differences observed when a comparison was made between methods of delivery were attributable to a decrease in $AUC_{0-\text{last}}$ when DL- α -lipoic acid was included as an ingredient in an extruded food. A similar pattern was observed for the 2.5 mg/kg dosage as in the 12.5 and 25 mg/kg dosages; the largest $AUC_{0-\text{last}}$ value was calculated from data from dogs orally administered DL- α -lipoic acid in a capsule form without a meal followed respectively by the capsule form provided with a meal and finally when included as an ingredient in an extruded dog food. The oral administration of DL- α -lipoic acid in a capsule form and provided with a meal resulted in a 51% decrease in the $AUC_{0-\text{last}}$ at the 2.5 mg/kg dosage. However, calculations for $AUC_{0-\text{last}}$ at the 2.5 mg/kg dosages were based on many results of DL- α -lipoic acid in plasma concentrations that were below the level of quantitation, especially when DL- α -lipoic acid was included as an ingredient in an extruded food; therefore, this may have resulted in an artificially decreased $AUC_{0-\text{last}}$ for this dosage group.

T_{\max} —There was a significant effect of method of administration on T_{\max} only at the 2.5 mg/kg dosage where the greatest time value was observed for the α -lipoic acid when it was included as an ingredient in an extruded dog food, compared with the oral administration of α -lipoic acid in a capsule form and provided with or without a meal. There was no significant difference detected at the 12.5 or 25 mg/kg dosages.

Discussion

Analysis of the results of the study reported here revealed that the pharmacokinetic parameters of DL- α -lipoic acid were affected by both dosage and method of administration. Absorption is decreased when DL- α -lipoic acid is included as an ingredient in an extruded food, compared with absorption of a comparable dosage of DL- α -lipoic acid when administered orally in a capsule form and provided with or without a meal. Minimal and mostly nonsignificant differences were detected between reported pharmacokinetic parameters when DL- α -lipoic acid was administered orally in a capsule form and provided with or without a meal. Most of the significantly different results detected were because of the comparatively reduced absorption of DL- α -lipoic acid from all 3 of the specially formulated extruded dog foods. The present study also revealed that plasma concentrations of α -lipoic acid increased in proportion with the dosage administered, regardless of the method of administration.

The C_{\max} for enantiomerically pure R- α -lipoic acid in humans varies from 400 to 1,150 ng/mL when 1,000 mg of R- α -lipoic acid is administered orally.^{1,3,7,16,k,1} A C_{\max} of 5,000 and 10,000 ng/mL has been reported¹⁷ for 300 and 600 mg of R- α -lipoic acid when administered as the tromethamine salt, respectively. A C_{\max} of 16 g/mL was observed when 600 mg of R- α -lipoic acid was administered orally to humans.¹⁷ When 600 mg was administered orally to the same individual, the C_{\max} of the sodium salt form of R- α -lipoic acid was 25.86 times as high as the C_{\max} observed for the aqueous form of R- α -lipoic acid.¹⁸ The oral administration of a 2.6 mg of a racemic mixture of RS- α -lipoic acid/kg dosage in a capsule to humans resulted in a C_{\max} of 1.95 and 1.17 g/mL for the R and S forms, respectively.⁹ Body weights of the humans in these studies^{9,17,18} were not reported; however, if a 70-kg body weight is used, an approximate oral dosage of 8.6 to 14.3 mg of α -lipoic acid/kg would result from the intake of 600 to 1,000 mg of α -lipoic acid. These dosages of α -lipoic acid are within the range of dosages for oral administration to dogs in the present study and resulted in comparable values for C_{\max} (C_{\max} range, 47 ng/mL [2.5 mg of DL- α -lipoic acid/kg dosage that was included in an extruded dog food] to 5,441 ng/mL [25 mg of α -lipoic acid/kg dosage in a capsule form and provided with a meal]). The variance in the values for C_{\max} in humans may be attributable to the forms of α -lipoic acid administered, methods of quantitation, and differences in fasting or withholding food before the administration of α -lipoic acid. The oral administration of α -lipoic acid followed by ingestion of fat 100 minutes later results in a second peak in the plasma concentration of α -lipoic acid and indicates that ingestion of fat enhances absorption of α -lipoic acid after an initial administration.¹ Thus, food, nutrient profile of a food, and time of food ingestion may influence the plasma concentration of α -lipoic acid detected.

The C_{\max} of α -lipoic acid for the dogs in the study reported here was within the range of values reported¹⁸ for other species. Analysis of results of the present study revealed that concentrations of DL- α -lipoic

acid in plasma increased with increasing dosages of DL- α -lipoic acid for each method of delivery. When DL- α -lipoic acid was administered as a capsule, the concomitant administration of food generally reduced concentrations in plasma. An exception to this was increased concentrations for dogs administered a 25 mg of α -lipoic acid/kg dosage in a capsule form that was concomitantly administered with a meal. The reasons for this phenomenon remain unknown, but they could have been related to incomplete dosage administration, errors during analysis, or some other technical abnormality. However, because the differences between the mean values for the C_{\max} of orally administered DL- α -lipoic acid when in a capsule form and provided with or without a meal were not significant, the technical issues of the events that led to this may not be important. The real difference also may have been related to the variability of C_{\max} among dogs.

The T_{\max} of orally administered DL- α -lipoic acid ranged between 21.7 minutes (2.5 mg/kg dosage that was in a capsule form and provided without a meal) to 105 minutes (2.5 mg/kg dosage that was included as an ingredient in an extruded food). This range of T_{\max} values approximates that reported^{1,17} in other animal species. In plasma, α -lipoic acid is rapidly absorbed into cells and converted to dihydro- α -lipoic acid. α -Lipoic acid is partitioned in plasma between the protein-bound and unbound forms. Most analytic methods detect total α -lipoic acid in plasma and do not distinguish between protein-bound and unbound forms. Movement of α -lipoic acid from plasma is likely the result of cellular absorption, metabolism into other metabolites, or elimination from the vascular space by renal or hepatic mechanisms.²

The λ_z of α -lipoic acid from plasma was similar among the dosages administered orally in a capsule form and provided with or without a meal but less than when α -lipoic acid was included in an extruded dog food. The $T_{1/2}$ of DL- α -lipoic acid when administered orally in a capsule form and provided with or without a meal was similar to that of other species.^{1,2,7,17,18} Despite the longer $T_{1/2}$ when included in an extruded dog food, a reduction in the AUC_{0-last} of DL- α -lipoic acid was attributable to a lower C_{\max} . The longer $T_{1/2}$ observed in dogs fed the extruded foods may have been attributable to slower absorption from the gastrointestinal tract because of the complex matrix used to formulate the extruded foods.

Pharmacokinetic parameters of DL- α -lipoic acid when administered orally to the dogs of the present study were significantly affected by whether a capsule or extruded dog food was used to provide DL- α -lipoic acid and, to a lesser extent, whether DL- α -lipoic acid was administered in a capsule form and was provided with a meal. The inclusion of DL- α -lipoic acid as an ingredient in an extruded dog food significantly decreased C_{\max} and delayed T_{\max} , compared with C_{\max} and T_{\max} when DL- α -lipoic acid was administered orally to dogs in a capsule form and provided with or without a meal following withholding of food for 12 hours. The pharmacokinetic parameters of DL- α -lipoic acid when administered orally in capsules were minimally affected by the fed status; furthermore, these parameters were

similar when compared with the results of other species. In addition, the plasma concentration of α -lipoic acid was proportional to the dosage administered, regardless of whether it was administered orally in a capsule form or included as an ingredient in an extruded dog food.

- a. Hill's Pet Nutrition Inc, Topeka, Kan.
- b. DSM Nutritional Products Inc, Fort Worth, Tex.
- c. Eurofins Scientific Inc, Des Moines, Iowa.
- d. Torpac Inc, Fairfield, NJ.
- e. Agilent 1100 HPLC, Agilent Technologies, Wilmington, Del.
- f. Supelcosil LC-18-DB analytical column, Supelco, Bellefonte, Pa.
- g. Supelguard LC-18-DB guard cartridge, Supelco, Bellefonte, Pa.
- h. ChemStation for LC 3D, rev. B.01.01, Agilent Technologies, Wilmington, Del.
- i. WinNonlin, version 4.1, Pharsight Corp, Mountain View, Calif.
- j. SAS, version 9.1, SAS Institute Inc, Cary, NC.
- k. Haenen GR, Bast A, Stolk L. Involvement of the metabolites in the antioxidant effect of lipoic acid. Proceedings of the Dutch Society of Clinical Pharmacology and Biopharmacy Meeting of October 4, 2005 (abstr). *Br J Clin Pharm* 2005;480/61.4.
- l. Krone D. *The pharmacokinetics and pharmacodynamics of R-(+)- α -lipoic acid*. PhD dissertation, Johann Wolfgang Goethe University, Frankfurt am Main, Germany, 2002.

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Correction: Evaluation of peptide- and recombinant protein-based assays for detection of anti-*Ehrlichia ewingii* antibodies in experimentally and naturally infected dogs

In this report, published October 2010 (*Am J Vet Res* 2010;71:1195–1200), the title was printed in error and should appear as follows: Evaluation of peptide- and recombinant protein-based assays for detection of anti-*Ehrlichia ewingii* antibodies in experimentally and naturally infected dogs