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Pharmacokinetics of multivesicular liposomal encapsulated cytarabine when administered subcutaneously in dogs

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Abstract

Background: Prolonged cytotoxic concentrations of cytarabine (CA) are required for maximum cytotoxicity. DepoCyt is a human liposomal cytarabine (LC) product that lasts longer in plasma and CSF compared with free CA (FC). The use of LC has not been evaluated in dogs.

Objectives: To perform a LC pharmacokinetic (PK) study when administered SC in dogs.

Animals: Five healthy female beagles.

Methods: Three-period, 3-treatment, nonblinded, randomized, and crossover design, including a pilot study. LC was administered at 50 mg/m^2 SC and FC was administered at 25 and 50 mg/m^2 SC and IV. Plasma CA concentrations were measured until 240, 72, and 8 hours after SC LC, SC FC, and IV FC administration, respectively. CA plasma concentrations were quantitated by ultra-high-performance liquid chromatography with mass spectrometry (MS/MS) detection and concentration-time profiles were evaluated by noncompartmental analysis.

Results: Subcutaneous LC administration resulted in a maximum plasma concentration of 26.3 to 59.78 ng/mL, time to reach maximum plasma concentration of 2 hours, area under the concentration-time curve to last measurable concentration of 669.3 to 1126 h × ng/mL, and plasma bioavailability (%F) of 19.6% to 31.3%. The PK profiles of FC after SC and IV administration differed when compared with LC.

Conclusions and Clinical Importance: In healthy dogs, SC LC administration at 50 mg/m^2 results in measurable plasma CA concentrations, is apparently safe and well tolerated, but does not result in prolonged cytotoxic plasma concentrations. Poor absorption of LC prevented establishment of a complete LC PK profile.

Abbreviations: λz, terminal first-order rate constant; ara-CTP, arabinosylcytosine triphosphate; AUC_{∞}, area under the concentration-time curve to infinity; AUC_{last}, area under the concentration-time curve to last measurable concentration; AU-CVMcblood sample—BS, Auburn University College of Veterinary Medicine; b-CSF, blood-cerebrospinal fluid; BUN, blood urea nitrogen; CA, cytarabine; CBC, complete blood count; CL/F, clearance (CL) or apparent clearance; C_{max}, maximum plasma concentration; CNS, central nervous system; CRI, constant rate infusion; DNA, deoxyribonucleic acid; FC, free cytarabine; IT, intrathecal; LC, liposomal cytarabine; MRT, mean residence time; MUE, meningoencephalomyelitis of unknown etiology; PK, pharmacokinetic; PS, pilot study; SP, study period; $t_{½}$, terminal half-life; THU, tetrahydrouridine; T_{max}, time to reach maximum plasma concentration; Vz or Vz/Fc, apparent volume of distribution.

KEYWORDS
canine, chemotherapy, cytarabine, cytosar, DepoCyt, leukemia, lymphoma, meningoencephalomyelitis of unknown etiology, pharmacokinetics

1 | INTRODUCTION

The antimetabolite chemotherapeutic drug 1-arabinofuranosylcytosine (*molecular formula*: $C_9H_{13}N_3O_5$, *CAS RN*: 147-94-4), known as cytarabine (CA), is an s-phase specific nucleoside analogue used in protocols for the treatment of meningoencephalomyelitis of unknown etiology (MUE) and lymphoproliferative disorders of the central nervous system (CNS), as it can penetrate the blood-cerebrospinal fluid (b-CSF) barrier and interrupt the lymphocyte cell cycle.[1-11]

Because CA is cell-cycle-specific, prolonged cytotoxic concentrations (≥100 ng/mL) in plasma and CSF are critical for maximum cytotoxicity.[10,12] Previous pharmacokinetic (PK) studies have evaluated CA administered to healthy dogs.[8,13] In these studies, administration as an IV bolus, SC injection, or CRI (constant rate infusion), resulted in plasma elimination half-lives ($t_{½}$) of 1.15, 1.35, and 1 to 1.15 hours, respectively.[8,13] The $t_{½}$ in CSF was 1.8 and 2.75 hours after SC injection and CRI discontinuation, respectively.[8] In addition to demonstrating that CA is eliminated more slowly from CSF when compared to plasma, these studies also demonstrated that only administration as a CRI results in steady-state cytotoxic concentrations for the duration of the infusion in both plasma and CSF.[8,13] The PK parameters of CA when administered SC to dogs with MUE is similar to those obtained in healthy dogs.[14]

CA administered as a CRI requires increased time, expense, and hospital admission. Often, owners are unable to afford the costs or are unwilling to hospitalize their pets. Therefore, an alternative route of administration is needed. Liposomal cytarabine (LC) may offer an alternative to free cytarabine (FC) administration. DepoCyt (Pacira Pharmaceuticals, San Diego, California) is a recently discontinued LC product labeled for intrathecal (IT) administration in humans with neoplastic meningitis.[12,15-17] Previous PK studies evaluating the use of LC in humans, rodents, and rhesus monkeys have demonstrated that it results in longer plasma and CSF $t_{½}$ when compared to FC.[12,15-20] In humans, when LC is administered IT, the CSF $t_{½}$ is 2 weeks, compared with a few hours when FC is used.[12,15-17] In rats, IT administration of LC results in a CSF $t_{½}$ of 148 hours, compared to 2.7 hours when FC is used.[18] In rhesus monkeys, IT administration of LC results in a CSF $t_{½}$ of 156 hours, compared with 0.74 hours when FC is used.[20] Last, evaluation of SC administration of LC in mice demonstrated that the CSF drug $t_{½}$ was 4 days, compared to 10 minutes when FC was used.[19] In dogs, to our knowledge, the use of LC when administered IT or SC has not been evaluated.

The objectives of this pilot study (PS) are to perform a PK analysis of LC when administered SC in dogs and compare the findings to the PK of FC when administered SC and as an IV bolus.

2 | MATERIALS AND METHODS

2.1 | Animals

The experimental methods and design utilized in this study were reviewed and approved by the Institutional Animal Care and Use Committee (Protocol #2017-3024) at Auburn University College of Veterinary Medicine (AU-CVM). A total of 5 healthy laboratory-acquired beagle dogs were utilized in the study. All dogs were intact females, 1 year old, and ranged in weight from 6.2 to 9.2 kg. Before initiation of the study, all dogs were deemed healthy via a physical exam, CBC, blood urea nitrogen (BUN), creatinine, and urine specific gravity (USG). At the end of the study, all dogs were spayed and adopted after an animal adoption agreement was signed by both the adopter and designated Project Veterinarian from the Division of Laboratory Animal Health at AU-CVM.

2.2 | Study design and overview

This study utilized a 3-period, 3-treatment, nonblinded, and randomized crossover design, which included a PS. The PS was performed during the first study period (SP), where dogs received SC LC with the goal of establishing optimal blood sampling times and dose. The PS was also performed with the goal of assessing any potential adverse reactions to LC and to establish the duration of time for which the dogs were considered to be actively excreting the drug.

A percutaneous double-lumen catheter (MILA International, Florence, Kentucky) was placed in each dog's jugular vein for blood sampling purposes the day before initiation of each SP and removed once the SP was completed. All dogs were sedated with dexmedetomidine (*chemical name*: (±)-4-[1-(2,3-dimethylphenyl)ethyl]-1H-imidazole monohydrochloride, *molecular formula*: $C_{13}H_{16},N_2$, *CAS RN*: 145108-58-3) at 12 μg/kg IV and butorphanol (*chemical name*: L-N-Cyclobutylmethyl-3, 14-dihydroxymorphinan tartrate salt, *molecular formula*: $C_{21}H_{29}NO_2$ $C_4H_6O_6$, *CAS RN*: 58786-99-5) at 0.3 mg/kg IV for catheter placement and were reversed with the appropriate dose of atipamezole (*chemical name*: 5-(2-ethyl-1,3-dihydroinden-2-yl)-1H-imidazole, *molecular formula*: $C_{14}H_{16}N_2$, *CAS RN*: 104054-27-5) IM once the catheter was properly placed and secured. All dogs had a CBC, BUN, creatinine, and USG performed before initiation of each SP, as well as 7 days after FC administration and 7 and 14 days after LC administration. Adverse effects were reported by the Common Terminology Criteria for Adverse Events guidelines.[21]

During the first SP, 2 dogs (dogs 1 and 2) were randomly selected as pilot dogs (PD) and received a SC injection of LC at 50 mg/m^2. The remaining dogs (dogs 3, 4, and 5) received a SC injection of FC at

25 mg/m^2. During the second SP, the PD received a SC injection of FC at 50 mg/m^2 and the remaining dogs received an IV bolus of FC at 25 mg/m^2. During the third and last SP, the PD received an IV bolus of FC at 50 mg/m^2. Because of discontinuation of DepoCyt production by Pacira Pharmaceuticals, it was not possible to treat all dogs in the study with LC as originally intended. A washout period of at least 7 days was used after administration of FC. A washout period of more than 3 months was used after administration of LC, which ultimately depended on when the PK analysis of the PD became available.

2.3 | Blood sampling times

A blood sample (BS) was collected in all dogs before administration of any formulation of CA at the beginning of each SP to ensure proper washout of the drug at time 0 and to examine background effects that could potentially alter results. After SC administration of LC, BS was collected 10 minutes and 2, 6, 12, 24, 36, 48, 72, 96, 120, 144, 168, and 240 hours after administration. After SC administration of FC, BS was collected 5, 15, and 30 minutes and 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 14, 18, and 72 hours after administration. After administration of FC as an IV bolus, BS was collected 5, 15, and 30 minutes and 1, 1.5, 2, 3, 4, 6, and 8 hours after administration. All BSs were collected in preiced heparinized tubes containing tetrahydrouridine (THU) to prevent conversion of ara-CTP to uracil arabinoside. Immediately after collection BSs were centrifuged for 10 minutes, and the plasma obtained was collected and stored in a freezer at −80°C until analysis of the samples was performed.

2.4 | Instrumentation

CA plasma concentrations were determined by ultra-high-performance liquid chromatography (UHPLC) with mass spectrometry (MS/MS) detection by a method described by Hillhorst et al, with some modifications.[22] An Agilent 1290 Infinity system (Agilent Technologies, Santa Clara, California) was employed, which consisted of a UHPLC system with cooling autosampler and column oven. An Agilent 6460 Triple Quadrupole LC/MS system (Agilent Technologies) was used for detection. Chromatographic separation was accomplished with a high strength silica T3 (100 × 2.1 mm internal diameter, 1.8 μm particles, Waters). All data were acquired and processed by using a commercially available software program (MassHunter Software, Agilent Technologies).

2.5 | HPLC and MS conditions

Gradient elution was applied for separation consisting of mobile phases A (0.1% formic acid in water) and B (0.1% formic acid in methanol) as follows: From 0% to 10% B from 0 to 2 minutes to 60% B at 4 minutes. The flow rate was set to 0.3 mL/min and the column was kept at 30°C.

2.6 | Preparation of stock solutions and samples

Solutions of CA and internal standard, cytarabine-$^{13}C_3$, were prepared by dissolving them in double-distilled water to produce stock concentrations of 1 mg/mL. Working solutions of CA and internal standard were prepared at a 100 and 10 μg/mL, respectively. All stock solutions were stored in amber vials, sealed, and kept at 4°C. Calibration standards (0-1000 ng/mL) were prepared by spiking appropriate stock into blank canine plasma containing THU at 50 μg/mL. Quality control samples (QC) were prepared from independently weighted stock solution of CA and internal standard, cytarabine-$^{13}C_3$ at CA concentrations of 1.00, 50.0, and 500 ng/mL. The intra- and interday accuracy and variation were determined (n = 3 different days). The linear range was 0.2 to 1000 ng/mL, with a limit of quantification of 0.2 ng/mL with accuracy of 90% to 110% and variation (CV%) <15%. The intra- and interday accuracies were within 90% to 110% and variation were less than 10% for all QC samples. Samples and standards were spiked with internal standard at 50 ng/mL.

Plasma samples and calibration standards were prepared by adding 15 μL of trifluoroacetic acid to 100 μL of plasma sample or standard and centrifuged at 1500*g* (relative centrifugal force) for 15 minutes. The supernatant was moved to clean tubes, dried under nitrogen stream, and reconstituted in 100 μL of 90:10 0.1% formic acid in water and acetonitrile. A volume of 90 μL was then transferred to a low volume insert in HPLC vials for injection.

2.7 | PK and statistical analysis

The LC and FC concentration-time profiles after IV bolus and SC administration were evaluated by noncompartmental PK analysis and modeling was performed by using the commercially available software program Phoenix WinNonlin version 8.0 (Phoenix 64 WinNonlin, Certara, Princeton, New Jersey). Pharmacokinetic parameters determined included maximum plasma concentration (C_{max}), time to reach maximum plasma concentration (T_{max}), area under the concentration-time curve (AUC) to last measurable concentration (AUClast), AUC to infinity (AUC∞), terminal first-order rate constant (λz), terminal half-life ($t_{½}$), clearance (CL) or apparent clearance (CL/F), apparent volume of distribution (Vz or Vz/F), and mean residence time (MRT).

Selection of terminal time points was based on the software choice and review of log-linear decline in postpeak plasma concentrations. The value of T_{max} was the observed time of highest plasma concentration and the value of C_{max} was the plasma concentration at that time. Area under the plasma concentration values were determined until the last time of a quantifiable plasma concentration (AUClast) by the linear/log trapezoidal rule and to infinity (AUC∞) based on the last plasma concentration and λz. Estimates of λz were calculated as the negative slope of the regression line for the terminal linear portion of the LN-transformed plasma concentration versus time curve. Estimates of $t_{½}$ were calculated as LN (2)/λz. CL or CL/F, where %F is the

percentage fraction absorbed, were both calculated as dose/AUC∞. Vz or Vz/F was estimated as CL/F divided by λz. MRT was calculated from the first moment of the AUC (AUMC) divided by AUC and the MRT for absorption (MAT) was obtained from the difference between MRTs for SC and IV injection dosing.

Comparison of CA PK parameters between doses, routes, and formulations were performed by analysis of variance (Phoenix 64 WinNonlin, Certara) with a significance level of $P < .05$. Parameters compared were CL, Vz, MRT, λz, and dose-adjusted values of C_{max} and AUC.

3 | RESULTS

The PK and statistical results from all dogs in the study after administration of FC and LC are summarized in Tables 1 and 2. Dog 5 was removed from the study because of behavioral problems and only received SC FC at 25 mg/m^2. No adverse events occurred in any of the dogs.

Analysis of variance methods were applied to compare effects of dose and dosage form on the parameters of CL, Vz, MRT, λz, and dose-adjusted values of C_{max} and AUC. No dose effects ($P > .05$) on PK parameters were noted after IV dosing. After SC administration of FC, there were no dose effects ($P > .05$) on CA PK parameters except that λz was greater at the higher 50 mg/m^2 dose. For SC versus IV at both doses administered, PK parameters were similar ($P > .05$) except C_{max}/Dose values that were greater as expected after IV administration.

After SC administration of LC at 50 mg/m^2, it was not possible to determine all of the PK parameters because of the low plasma concentrations obtained. All LC PK parameters were different ($P < .05$) when compared to FC PK parameters.

4 | DISCUSSION

The role of liposomal encapsulated chemotherapy drugs is not clearly established in veterinary medicine. The primary objective of this study was to determine the PK of LC after SC administration in dogs. The secondary objectives were to compare the PK of SC administered LC to the already established PK of FC when administered SC and IV in dogs. In this study, SC administration of LC in dogs resulted in measurable CA plasma concentrations. In addition, SC administration of LC to healthy dogs at a dose of 50 mg/m^2 appeared to be safe and well tolerated. The cytotoxic CA plasma concentration needed for effectiveness of the drug, however, was not achieved at a dose of 50 mg/m^2. The PK results obtained after SC and IV bolus administration of FC were similar to those obtained in previously published PK studies, but were different when compared to the PK profile of SC administered LC. [8,13,14] When comparing SC and IV FC PK results, CA appeared to be completely absorbed after SC administration.

As shown in Figure 1, the PK profile of SC LC was characterized by an initial rapid CA absorption from the liposomal product and a rapid peak in drug plasma concentration. This was followed by an almost equally rapid decline in the drug plasma concentration and subsequent slow and prolonged drug absorption, which ultimately resulted in a very low, but constant CA plasma concentration until it was last measured (AUC_{last};10 days). The highest C_{max} obtained was 59.7 ng/mL, which is half of the established cytotoxic concentration of CA in plasma. After administration, only 20% to 30% of the administered CA was absorbed from the liposomal product by the time the last BS was obtained. Because of incomplete drug absorption from the administration site over 72 hours and low concentration measured in plasma, it was not possible to establish the full PK profile of LC after SC administration as intended.

TABLE 1 Noncompartmental pharmacokinetics of free cytarabine in healthy dogs (n = 4) after administration of an IV bolus at 25 and 50 mg/m^2

Dose (mg/m^2)	Subject	C_{max} (ng/mL)	T_{max} (h)	AUClast (h × ng/mL)	AUCinf (h × ng/mL)	λz (1/h)	$t_{½}$ (h)	Cl (L/h/m^2)	Vss (L/m^2)	Vz (L/m^2)	MRT (h)
25	Dog 3	1437	0.083	1479	1531	0.5234	1.324	16.33	27.41	31.2	1.679
	Dog 4	1622	0.083	1104	1124	0.4959	1.398	22.25	29.18	44.86	1.311
	Mean	1530	0.083	1291	1327	0.5097	1.361	19.29	28.3	38.03	1.495
	CV%	8.6	0	20.5	21.7	3.8	3.8	21.7	4.4	25.4	17.4
	GeoMean	1527	0.083	1278	1312	0.5095	1.361	19.06	28.28	37.41	1.484
50	Dog 1	4298	0.083	3398	3413	0.6462	1.073	14.65	18.34	22.67	1.252
	Dog 2	3766	0.083	3583	3597	0.6718	1.032	13.9	18.62	20.7	1.339
	Mean	4032	0.083	3490	3505	0.659	1.052	14.28	18.48	21.68	1.296
	CV%	9.3	0	3.7	3.7	2.7	2.7	3.7	1.1	6.4	4.8
	GeoMean	4023	0.083	3489	3504	0.6589	1.052	14.27	18.48	21.66	1.295

Abbreviations: AUCinf, area under the concentration curve to infinity; AUClast, area under the concentration curve to last measurable concentration; CL, clearance; C_{max}, maximum plasma concentration; CV%, the percent coefficient of variation; GeoMean, the geometric mean; MRT, mean residence time; $t_{½}$, terminal plasma half-life; T_{max}, the time to reach maximum plasma concentration; Vss, steady-state volume of distribution; Vz, apparent volume of distribution; λz, terminal first-order rate constant.

TABLE 2 Noncompartmental pharmacokinetics in healthy dogs after SC administration of free cytarabine (FC) at 25 and 50 mg/m^2 (n = 5) and SC administration of liposomal cytarabine (LC) at 50 mg/m^2 (n = 2)

Dose (mg/m^2)	Subject	C_{max} (ng/mL)	T_{max} (h)	AUClast (h × ng/mL)	AUCinf (h × ng/mL)	λz (1/h)	$t_{½}$ (h)	Cl/F (L/h/m^2)	V/F (L/m^2)	MRT (h)	MAT (h)	%F
25	Dog 3	1118	0.25	1585	1586	0.5443	1.273	15.77	28.97	1.382	0.162	103.6
SC FC	Dog 4	1608	0.25	1998	2000	0.4043	1.714	12.5	30.92	1.53	0.219	178.9
	Dog 5	995.7	0.25	1464	1464	0.3954	1.753	17.07	43.18	1.559		
	Mean	1241[a]	0.25	1682	1683	0.448	1.58	15.11	34.35	1.491	0.191	141.2
	CV%	26.1	0	16.7	16.7	18.6	16.9	15.6	22.4	6.4	21.2	37.7
	GeoMean	1214	0.25	1667	1668	0.4431	1.564	14.99	33.82	1.489	0.188	136.1
50	Dog 1	2265	0.25	3784	3784	0.6474	1.071	13.21	20.41	1.519	0.267	110.9
SC FC	Dog 2	2351	0.5	3911	3911	0.8219	0.8434	12.78	15.55	1.522	0.183	108.7
	Mean	2308[a]	0.38	3847	3848	0.7346[b]	0.957	13	17.98	1.521	0.225	109.8
	CV%	2.6	47.1	2.3	2.3	16.8	16.8	2.3	19.1	0.1	26.4	1.4
	GeoMean	2308	0.35	3847	3847	0.7295	0.9502	13	17.82	1.521	0.221	109.8
50	Dog 1	59.78	2	669.3	770.3							19.6
SC LC	Dog 2	26.3	2	1126	1811							31.3
	Mean	43.04[c]	2[c]	897.4[c]	1291[c]							25.5[c]
	CV%	55	0	35.9	57							32.5
	GeoMean	39.65	2	868	1181							24.8

Abbreviations: %F, the percentage fraction absorbed; AUCinf, area under the concentration curve to infinity; AUClast, area under the concentration curve to last measurable concentration; CL/F, apparent clearance; C_{max}, the maximum plasma concentration; CV%, the percent coefficient of variation; GeoMean, the geometric mean; MAT, the mean absorption time; MRT, mean residence time; $t_{½}$, terminal plasma half-life; T_{max}, the time to reach maximum plasma concentration; V/F, apparent volume of distribution; λz, terminal first-order rate constant.

[a]Statistically significant difference ($P < .05$) between IV and SC routes by dose.

[b]Statistically significant difference ($P < .05$) between 25 and 50 mg/m^2 SC doses.

[c]Statistically significant difference ($P < .05$) between liposomal and nonliposomal cytarabine at a dose of 50 mg/m^2.

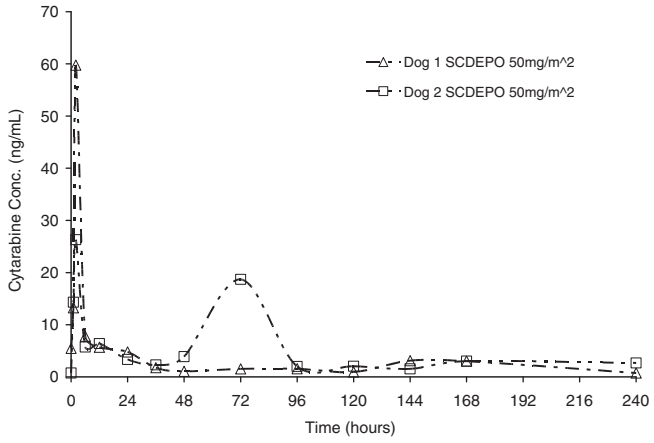

FIGURE 1 Cytarabine concentration-time profiles after SC administration of liposomal cytarabine (SCDEPO, LC) with a dose of 50 mg/m^2 in healthy dogs (N = 2). The time is shown on the *x*-axis and cytarabine concentration on the *y*-axis

When comparing the PK profile of SC administered LC and FC, there were several important differences noted. Although both drug formulations were rapidly absorbed after administration and demonstrated a rapid peak in CA plasma concentrations, the T_{max} obtained was much higher for LC than for FC (2 versus 0.25 hours, respectively). When comparing the C_{max} and AUC obtained, the dose used for LC (50 mg/m^2) did not result in achievement of cytotoxic plasma concentrations, as opposed to the doses used for FC (25-50 mg/m^2), which did achieve cytotoxic plasma concentrations. As shown in Figures 1 and 2 and as evidenced by the $t_{½}$ obtained for FC, the concentration of both drugs rapidly decreased after the C_{max} was reached. A major difference was that although the plasma concentration of CA was negligible 6 hours after FC administration, there was still measurable CA in plasma 240 hours after LC administration (<5 ng/mL). Lastly, while 100% of the given FC dose was absorbed, only 20% to 30% of the given LC dose was absorbed by the time the last BS was obtained (Figure 3).

The differences between PK profiles found in this study can mainly be attributed to the pharmacological characteristics of liposomal encapsulated products, which allow for a slow-release drug delivery system.[16] Liposomes are spherical lipid-based nanoparticles that have a bilayer membrane composed of natural and/or synthetic phospho- and sphingo-lipids, along with other membrane constituents, such as cholesterol.[16,23] They are hydrophilic on the inside and outside, and have an external hydrophobic wall.[24] Although their molecular composition makes them ideal carriers for SC administered drugs, it is not clear why there was incomplete CA absorption from

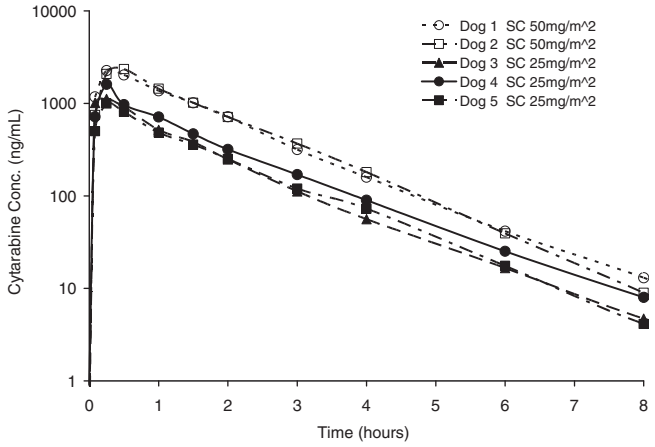

FIGURE 2 Cytarabine concentration-time profiles after SC administration of free cytarabine with a dose of 25 mg/m^2 and 50 mg/m^2 in healthy dogs (n = 5). The time is shown on the *x*-axis and cytarabine concentration on the *y*-axis

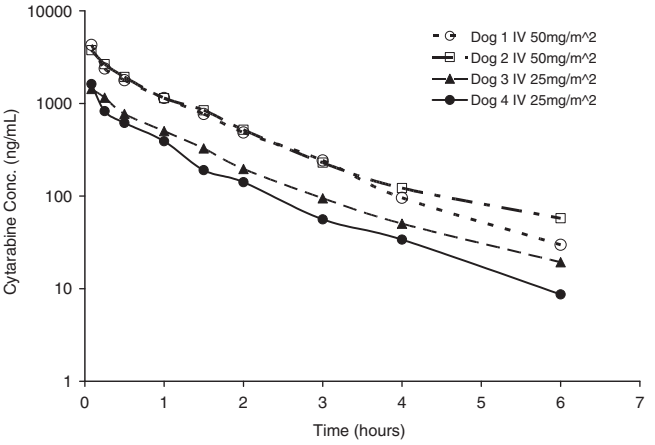

FIGURE 3 Cytarabine concentration-time profiles after IV administration of free cytarabine with a dose of 25 mg/m^2 and 50 mg/m^2 in healthy dogs (n = 4). The time is shown on the *x*-axis and cytarabine concentration on the *y*-axis

the liposomal product in this study. Possible explanations include drug metabolization at the injection site, slow drug release from the liposomal product, and factors related the liposomal nanoparticle itself, such as morphology, size, surface charge, phase transition temperature, and protein interactions, as these can all affect the delivery and absorption of the drug.[16]

The drug doses used in this study are also in part responsible for the differences noted between the PK profiles obtained. Although the FC doses used resulted in achievement of cytotoxic plasma concentrations, the $t_{½}$ was very short when the drug was administered by both the SC and IV bolus routes. Because of the short $t_{½}$ and established mechanism of action, exposure to prolonged cytotoxic concentrations is critical if maximum cytotoxicity is to be achieved; therefore, SC and IV bolus administered FC likely represent ineffective ways to use this drug. Based on the C_{max} obtained after SC administration of LC, doubling the dose used would have likely resulted in achievement of cytotoxic plasma concentrations. However, because it was not possible to determine the $t_{½}$ of SC administered LC, it is not possible to predict if with a higher dose would result in an effective way to use this drug.

At the dose used in this study, LC appears to be safe and well tolerated when administered SC to healthy dogs. In humans, LC is administered IT for the treatment of lymphomatous meningitis. In this study, LC was only administered SC. In humans, the toxic adverse effects associated with IT administration of LC are similar to those seen when FC is used and include arachnoiditis, fever, headache, weakness, lethargy, nausea, and vomiting.[15-16] These are dose-dependent, mostly reversible, transient, and of mild to moderate severity.[15-16] The dogs utilized in this study were closely monitored during the complete duration of SP via daily physical exams, blood work, and urine analysis. No adverse effects or adverse reactions were observed during the SP.

The biggest limitation in this study was the small sample size of dogs that received SC LC, which ultimately prevented statistical analysis of the data obtained. The original study design accounted for administration of LC to all dogs enrolled and for adjustment of blood sampling times and doses used after completion of the PS. However, completion of the study as intended was not possible because of discontinuation of LC production by the pharmaceutical company during the course of this study.

Lastly, another limitation of the study was the inability to completely establish the PK profile of SC administered LC.

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CONFLICT OF INTEREST DECLARATION

Authors declare no conflict of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

The experimental methods and design utilized in this study were reviewed and approved by the IACUC at Auburn University College of Veterinary Medicine (Protocol #2017-3024).

HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for this study.

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