Phase I Trial of Doxorubicin-Containing Low Temperature Sensitive Liposomes in Spontaneous Canine Tumors

Marlene L. Hauck,[1] Susan M. LaRue,[2] William P. Petros,[3] Jean M. Poulson,[4] Daohai Yu,[5] Ivan Spasojevic,[6] Amy F. Pruitt,[1] Allison Klein,[1] Beth Case,[1] Donald E. Thrall,[1] David Needham,[7] and Mark W. Dewhirst[4]

Abstract

Purpose: To determine the maximum tolerated dose, dose-limiting toxicities, and pharmacokinetic characteristics of doxorubicin encapsulated in a low temperature sensitive liposome (LTSL) when given concurrently with local hyperthermia to canine solid tumors.

Experimental Design: Privately owned dogs with solid tumors (carcinomas or sarcomas) were treated. The tumors did not involve bone and were located at sites amenable to local hyperthermia. LTSL-doxorubicin was given (0.7-1.0 mg/kg i.v.) over 30 minutes during local tumor hyperthermia in a standard phase I dose escalation study. Three treatments, given 3 weeks apart, were scheduled. Toxicity was monitored for an additional month. Pharmacokinetics were evaluated during the first treatment cycle.

Results: Twenty-one patients were enrolled: 18 with sarcomas and 3 with carcinomas. Grade 4 neutropenia and acute death secondary to liver failure, possibly drug related, were the dose-limiting toxicities. The maximum tolerated dose was 0.93 mg/kg. Other toxicities, with the possible exception of renal damage, were consistent with those observed following free doxorubicin administration. Of the 20 dogs that received $\geq$2 doses of LTSL-doxorubicin, 12 had stable disease, and 6 had a partial response to treatment. Pharmacokinetic variables were more similar to those of free doxorubicin than the marketed liposomal product. Tumor drug concentrations at a dose of 1.0 mg/kg averaged 9.12 $\pm$ 6.17 ng/mg tissue.

Conclusion: LTSL-doxorubicin offers a novel approach to improving drug delivery to solid tumors. It was well tolerated and resulted in favorable response profiles in these patients. Additional evaluation in human patients is warranted.

Liposome-encapsulated chemotherapy was developed to improve selectivity of drug for tumor compared with normal tissue. Despite the achievement of tumor drug levels that are up to 10-fold higher than those achieved with unencapsulated drugs, particularly when given concurrently with hyperthermia, clinical efficacy of these agents has been only modestly improved (1–4). Decreased toxicity, in particular the cardiotoxicity seen with doxorubicin, has been the most significant benefit derived from these formulations.

One potential explanation for the lack of clinical benefit from liposomal drug delivery to the tumor tissue is the dependence of cytotoxicity on the presence of free drug. Although the liposomes may accumulate preferentially in tumors, the mechanisms by which traditional liposomes release their contents are not well understood (5). The development of liposomes engineered for triggered drug release is one approach that addresses this problem directly.

The development of hyperthermia-mediated drug release from liposomes was first reported in 1978 (6). These early thermosensitive liposomes typically released their contents at temperatures > 42°C. Temperatures in this range are difficult to achieve uniformly in a clinical setting. In addition, drug release was slow, requiring 30 minutes to release 40% of the contents (7). Because of these disadvantages, low temperature sensitive liposomes (LTSL) were developed to release their contents at temperatures from 39.5°C to 41.5°C. In contrast to the former thermosensitive formulations, LTSL release 100% of their contents within 20 seconds at the transition temperature of 41.3°C (5, 8).

LTSL containing doxorubicin were tested for antitumor efficacy when combined with 42°C heat in a murine xenograft model of squamous cell carcinoma and compared with free drug, nonthermally sensitive, and traditional thermally sensitive doxorubicin-containing liposome formulations. Although all three liposomal formulations resulted in a significant tumor growth delay compared with untreated controls and the group

Authors' Affiliations: [1]College of Veterinary Medicine, North Carolina State University, Raleigh, North Carolina; [2]College of Veterinary Medicine, Colorado State University, Fort Collins, Colorado; [3]Mary Babb Randolph Cancer Center, West Virginia University, Morgantown, West Virginia; and Departments of [4]Radiation Oncology, [5]Biostatistics and Bioinformatics, [6]Pharmacology, and [7]Biomedical Engineering, Duke University Medical Center, Durham, North Carolina
Received 1/31/06; revised 4/26/06; accepted 4/28/06.

Grant support: Department of Health and Human Services grant P01CA42745, NIH, Celsion Corp., and the Mylan Chair of Pharmacology at West Virginia University. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Requests for reprints: Marlene L. Hauck, Department of Clinical Sciences, College of Veterinary Medicine, North Carolina State University, 4700 Hillsborough Street, Raleigh, NC 27606. Phone: 919-513-8274; Fax: 919-513-6336; E-mail: marlene_hauck@ncsu.edu.

doi:10.1158/1078-0432.CCR-06-0226

treated with free drug, only the LTSL resulted in local control of all tumors at 60 days posttreatment (9). With the LTSL, there was a >25-fold increase in tumor doxorubicin levels compared with free drug.

Based on these promising results from a rodent trial, there may be great value in defining a LTSL treatment strategy for some human cancers. Before human trials can be defined, however, more information is needed on the toxicity and drug pharmacokinetic variables of LTSL therapy. In this report, we present the results of a phase I clinical trial of LTSL-encapsulated doxorubicin given to dogs with solid tumors, in conjunction with local hyperthermia, every 21 days. The objectives of this study were to identify the maximum tolerated dose (MTD) of LTSL-encapsulated doxorubicin, to determine the incidence and severity of toxicoses associated with LTSL-doxorubicin administration with local hyperthermia and to characterize the pharmacokinetics of LTSL-doxorubicin in dogs to facilitate the introduction of this novel approach to drug delivery in human patients.

Materials and Methods

Patient selection. Privately owned dogs with histologically diagnosed, and macroscopic soft tissue tumors (sarcomas or carcinomas) were eligible for inclusion in the phase I trial. Tumors were located at sites technically accessible for the administration of local hyperthermia with a spiral microwave applicator operating between 140 and 433 MHz (8). Inclusion criteria included a life expectancy of at least 6 months and patients that were not medically debilitated. Dogs with tumors, which had been previously irradiated or had bone involvement, were excluded. There was no exclusion for tumor size, metastatic disease, or previous (non-doxorubicin) chemotherapy or surgery. Informed consent was obtained from the owner for each patient, and the Institutional Animal Care and Use Committees at North Carolina State University, Colorado State University, and Duke University approved the protocol.

Study design. This was a phase I study of doxorubicin-containing LTSLs given with local hyperthermia, evaluating acute toxicity, defining the MTD, and determining the pharmacokinetics of the drug delivery system. The protocol included the administration of three treatments of LTSL-doxorubicin at 3-week intervals to each patient. After completing a 1-month posttreatment evaluation, patients went on to receive further treatment as appropriate for their tumor. Withdrawal from the study occurred if there was a 25% increase in the tumor volume or an increase in patient morbidity. A minimum of three patients per dose level were planned.

Dose escalation. A modified Fibonacci approach was employed to select dose levels (10). A starting dose of 0.7 mg/kg was used, based upon the published MTD for doxorubicin and Doxil in dogs (11, 12). The initial dose escalation was to 0.93 mg/kg, and, due to toxicities seen at this dose, the final dose escalation was to 1.0 mg/kg. The LTSL formulation was given i.v. over 30 minutes in all patients except the first two. No intrapatient dose escalations were done. Intrapatient dose reductions were permitted if a dose-limiting toxicity (DLT) was encountered.

Liposomes. LTSL were prepared by the lipid film hydration and extrusion method (13). The molar ratio of 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine/1-palmitoyl-2-hydroxy-*sn*glycero-3-phosphocholine/1,2-distearoyl-*sn*glycero-3-phosphoethanolamine-*N*-polyethylene glycol 2000 was 90:10:4. The mean diameter of liposomes used in this study was 100 nm.

Liposome-encapsulated doxorubicin. The LTSL-doxorubicin formulation was prepared with a ratio of doxorubicin to lipid of 0.2:1 (w/w). The LTSLs were prepared as a sterile formulation by the British Columbia Cancer Agency (certified Good Manufacturing Practices laboratory) for each patient and shipped on ice via overnight mail to arrive the day before treatment. Doxorubicin was encapsulated into the lipid vesicles using a pH gradient–driven loading protocol (14). After preparation, the doxorubicin-LTSL was kept at 4°C until ~30 minutes before use, at which time the formulation was allowed to equilibrate to room temperature. Before administering the drug, a 100-μL aliquot was tested for the presence of free doxorubicin with a colorimetric assay. To the aliquot of doxorubicin, 200 μL of normal saline and 200 μL of sodium carbonate (0.5 mol/L) was added. If free doxorubicin was not present (as assessed by no change in color), the calculated dose was given as a 30-minute i.v. infusion.

Pretreatment of patients. The first four dogs exhibited symptoms consistent with anaphylactoid reactions. Subsequent studies done in normal beagles revealed that the symptoms were due to liposome-mediated histamine release. This reaction also occurred with empty liposomes. Treatment with H_1/H_2 blockers and corticosteroids for 48 hours before therapy significantly decreased this reaction in the beagles (data not shown). Therefore, all subsequent tumor bearing dogs received 0.5 mg/kg of prednisone orally twice daily, 2 mg/kg of diphenhydramine orally thrice a day, and 0.5 mg/kg famotidine orally once a day, beginning 2 days before LTSL treatment. Thirty minutes before the infusion of the LTSLs, dogs received cimetidine (5 mg/kg i.v.), diphenhydramine (2 mg/kg i.m.), and dexamethasone $NaPO_4$ (2 mg/kg i.v.).

Hyperthermia administration. Dogs were anesthetized with inhalational isoflurane anesthesia. Thermometry probes (Luxtron 3100 Fluoroptic thermometer, Santa Clara, CA) were placed according to the Radiation Therapy Oncology Group guidelines using computed tomography imaging (15). The tumors were heated with a scanning spiral or miniature annular-phased array microwave applicator operating between 140 and 433 mHz until the T_{90} (lowest 10th percentile of temperatures) was 40.1°C, or the T_{50} (median temperature) reached 44°C (8, 16). An additional constraint was that normal tissue temperatures were not allowed to exceed 43°C. The limit for median temperature was based on prior observations that higher temperatures caused reduction in tumor pO_2, presumably from reduced perfusion as a result of vascular damage (17). Deionized water was used as the coupling medium. Thermometers were withdrawn either manually or automatically along the catheter track in 0.5-cm increments, and the temperatures were recorded every 6 minutes. Tumor heating was maintained at steady state during the 30 minutes of the constant-rate i.v. infusion of the LTSL-doxorubicin, and hyperthermia was continued for 1 hour after the infusion ended.

Plasma and tissue collection. Plasma samples for pharmacokinetic evaluation of the LTSL-doxorubicin were drawn at time 0 (before infusion), immediately after completion of the infusion, and at 15, 30 minutes, 1, 2, 3, 4, 8, 24, and 48 hours after infusion. Four milliliters of blood were drawn into an EDTA-containing vacutainer tube (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ), and the tube was immediately placed on ice. The blood was centrifuged at ~2,000 × *g* for 10 minutes at 4°C within 2 hours of collection. The plasma was removed and stored in at −70°C until analysis.

Tumor biopsies were obtained at 24 hours after drug administration. Biopsy samples used to determine the tumor doxorubicin levels were immediately frozen in liquid nitrogen and stored at −80°C until time of analysis. In addition, plasma and tumor specimens were collected in a subset of subjects on subsequent cycles.

Microdialysis. Doxorubicin concentration in tumor extracellular fluid was evaluated by a microdialysis technique. These procedures were done using commercially available microdialysis tubing with a membrane length of 10 mm, a molecular weight cutoff of 100,000 Da, and an outer diameter of 0.5 mm (model no. 830-9571, CMA Microdialysis, Solna, Sweden). The microdialysis probes were prepared and placed as previously described (18). When tumor size allowed, two probes were placed perpendicular to one another in different quadrants of the tumor. Relative recovery of the microdialysis tubing was calculated using urea as the recovery marker. The use of urea was

previously validated in a rat model (18). Samples were collected for analysis every 10 minutes, starting at the beginning of the 30-minute LTSL infusion, for 2 hours. Doxorubicin concentrations were analyzed as described below, and the measured levels were corrected using the relative recovery calculation.

Doxorubicin measurements. Plasma, microdialysate, and tumor tissue sample concentrations were determined by a validated high-performance liquid chromatography method using standard fluorescence detection or laser-based detection (Picometrics, Ramonville, France), respectively (19). The lower limit of quantification was 0.1 μg/mL for the standard detector and 50 pg/mL with the laser-based detector.

Pharmacokinetic analysis. Pharmacokinetic variables were generated by noncompartmental analyses using a standard, two-stage approach (WinNonlin v 2.1, Pharsight, Inc., Mountain View, CA).

Toxicity evaluation. Initial clinical laboratory evaluations included a complete blood count, chemistry panel, urinalysis, chest radiographs, and other appropriate staging tests. All dogs were scheduled to have weekly complete blood counts done for the duration of the study and routine staging, serum chemistries, electrocardiograms, and echocardiography before each treatment and at the final evaluation. All toxicities were evaluated based upon the National Cancer Institute Common Toxicity Criteria, version 2.0. Minor modifications were made based upon physiologic differences between dogs and humans (differences in reference ranges).

DLTs. DLT was defined as a grade ≥4 hematologic toxicity, grade ≥3 diarrhea or vomiting that did not resolve after 7 days, grade ≥3 infection or hepatic toxicity that did not resolve after 7 days, grade ≥4 renal failure or grade ≥3 dermatologic toxicity that did not resolve in 14 days, as defined by the National Cancer Institute Common Toxicity Criteria, version 2.0.

Results

Patients and treatment. Twenty-one dogs were admitted onto the study at two sites (11 from North Carolina State University and 10 from Colorado State University) from April 2000 to August 2003. Median age was 11 years (range, 4-15 years); 8 were male (7 neutered and 1 intact) and 13 were female (12 neutered and 1 intact). Median weight was 30 kg (range, 12.6-46 kg; n = 21). Eighteen dogs had soft tissue sarcomas: three had carcinomas. One dog had a single pulmonary lesion identified at initial staging. No studies were done to confirm this nodule as metastatic disease. Median size of these tumors was 90.6 cm^3 (range, 3.1-1747.0 cm^3). These 21 dogs received a total of 53 cycles of LTSL/local hyperthermia (1 dog received 1 cycle, 8 dogs received 2 cycles, and 12 dogs received 3 cycles). Dose levels of 0.7 mg/kg (eight dogs: one dog received 0.67 mg/kg due to a production problem, no DLTs), 0.93 mg/kg (eight dogs, one dose-limiting neutropenia), and 1.0 mg/kg (five dogs, two DLTs) were given. In the initial two patients treated, the liposomes were infused over 5 and 16 minutes, resulting in a sudden transitory decrease in blood pressure and an increase in end inspiratory pressure. Increasing the infusion time to 30 minutes in the next two dogs treated did not eliminate the physiologic response. As noted above, after finding this effect in the initial four dogs treated, further evaluation was done in normal dogs. The histamine related toxicity was decreased using premedication, and all subsequent animals received this regimen.

The number of dogs treated in the dose cohort at 0.7 mg/kg was increased to eight after the initial four dogs, when the liposome infusion rate was altered, and premedication was instituted. One additional dog was treated at this dose level as one dog received a slightly lower dose than planned (0.67 mg/kg) due to production difficulties. The dose cohort at 0.93 mg/kg was increased to eight dogs due to one unexpected patient death (not considered to be treatment associated), one DLT (febrile neutropenia), and one additional patient treated at this dose level due to scheduling concerns. Early discontinuation of treatment protocol occurred for the following reasons: death or euthanasia (three dogs: one patient in each dose cohort); progressive disease (two dogs: both in 0.7 mg/kg cohort); cardiotoxicity, grade 2, not a DLT (one dog: in the 0.93 mg/kg cohort); and potential early renal toxicity (isosthenuria) grade 0, not a DLT (two dogs: both in the 1.0 mg/kg cohort). Twenty dogs received at least two treatments: one dog in the 0.93 mg/kg cohort died of non-neutropenic sepsis, possibly related to subclinical endocarditis, 1 week after the first treatment.

Hyperthermia treatment. T_{50} reflects the median temperature within a tumor. The median T_{50} achieved during all of the first hyperthermia treatments was 41.45°C (range, 37.8-42.8°C) in the 21 dogs. The median T_{50} achieved over all given hyperthermia treatments was 41.22°C (range, 37.38-44.03°C). T_{90} reflects the lower end of the temperature distribution and is the temperature that 90% of all points are greater than or equal to. The median T_{90} for the first treatment was 39.48°C (range, 36.13-40.76°C), and the median T_{90} for all hyperthermia treatments was 39.51°C (range, 36.13-41.48°C).

Table 1. Summary of toxicities by dose level

Toxicity	Dose level (mg/kg) and worst grade of toxicity					
	0.7 (n = 8)		0.93 (n = 8)		1 (n = 5)	
	1-2	3-4	1-2	3-4	1-2	3-4
Neutrophils	3		3	2 (2 DLTs)	4	1 (1 DLT)
Platelets	3	1	4	2	4	1
Alanine aminotransferase	4	1	7		3	1
Creatinine	1				1	
Hepatic necrosis						1 (1 DLT)
Gastrointestinal						
Cutaneous	1					
Cardiac	2		3			

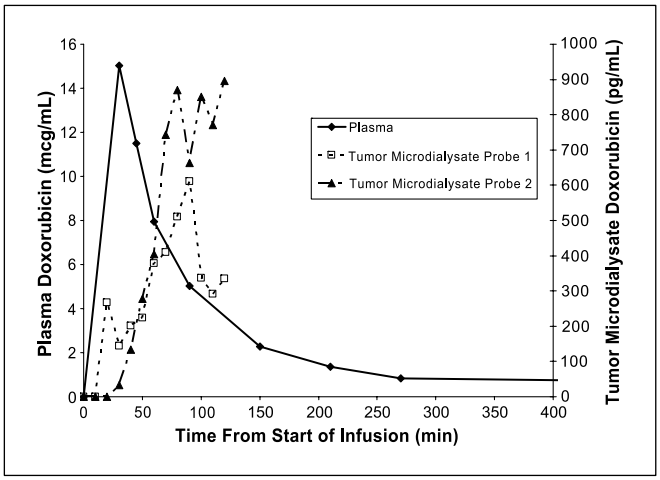

Fig. 1. Concentration-time profile for plasma and tumor microdialysate concentrations of doxorubicin in a representative canine patient who received the MTD of 0.93 mg/kg LTSL-doxorubicin and local hyperthermia.

DLT. Toxicities are presented in Table 1. Although there were two episodes of grade 4 neutropenia at the 0.93 mg/kg dose, one of these episodes was incorrectly entered into the database and missed until the final data review. This patient had a grade 4 neutropenia only after the second dose of the LTSL-doxorubicin and had no toxicity after the 1st dose. The dog was euthanized shortly after the third dose due to tumor necrosis; thus, no additional toxicity information is available. Two dogs had dose reductions: one was reduced to 0.6 mg/kg after the second treatment at 0.7 mg/kg due to acute renal toxicity following treatment. This dog was one of the first animals treated and was not premedicated for histamine prophylaxis. The renal toxicity was most likely a result of decreased renal perfusion secondary to histamine release. In one dog, the dose was reduced to 0.93 mg/kg for the third treatment due to grade 4 neutropenia following the second treatment at 1.0 mg/kg. Two DLTs were observed at 1.0 mg/kg: acute hepatic necrosis resulting in death and one grade 4 neutropenia (noted above). It is not possible to absolutely attribute the hepatotoxicity to the drug administration particularly because this patient did not have any elevations in liver enzymes following the first treatment. However, this patient also had the largest drug exposure, as measured by area under the curve (AUC). Histopathology of the liver revealed diffuse necrosis of unknown etiology.

Two dogs in the highest-dose group had therapy discontinued after the second treatment due to the presence of isosthenuria. Both dogs' renal disease progressed over the course of several months, resulting in euthanasia. Two dogs in the 0.93 mg/kg dose level also developed late renal failure: one 3 months after completing treatment and one over a year after completing the protocol.

No gastrointestinal toxicity was noted. Only one patient had cutaneous toxicity that was scored as a grade 2. Three patients died during treatment (2) or shortly following their last evaluation (1), and necropsies were done. One died from sepsis (non-neutropenic), presumably arising from subclinical endocarditis that was documented at necropsy; one dog died acutely from aspiration pneumonia, 1 week after the third treatment (non-neutropenic), also documented on necropsy; and one (in the 1.0 mg/kg cohort) from prolonged weakness and malaise following surgical resection of the tumor (1 month after the third treatment). Necropsy findings on this patient were suggestive of significant cardiotoxicity.

Necropsy data were available on five additional dogs as well. These dogs were euthanized at times ranging from 1 month following their last treatment to 1.5 years after treatment. In these five dogs, cardiac changes were reported as mild to moderate fibrosis in three; no evidence of cardiotoxicity in one; and in the last dog, the heart was not evaluated due to the presence of generalized lymphosarcoma. A variety of renal lesions were present involving both the glomeruli and tubules: most were consistent with the advanced age of these patients. The most common hepatic change (centrilobular degeneration, dropout, and atrophy) was present in two dogs. The remaining three dogs did not have consistent hepatic changes.

The maximum grade of toxicity experienced by each patient was compared with their AUC, and the maximum core temperature was recorded during the infusion and 1 hour post-infusion local hyperthermia treatment. The Spearman correlation coefficient was used to determine if there was any correlation between toxicity and AUC or maximum core temperature, and no significant association between toxicity and either AUC or maximum core temperature was found.

Pharmacokinetic results. Plasma doxorubicin concentrations were maximal at the end of the infusion and decreased relatively quickly thereafter (representative data in Fig. 1). Systemic doxorubicin exposure increased, on average, with dose level (Fig. 2; Table 2), and there was no evidence for accumulation between cycles (data not shown). The mean plasma clearance of doxorubicin was 0.43 L/h/kg but varied widely between subjects (Table 2). Similarly, the average terminal plasma half-life was 1.6 hours with a range of 0.13 to 6.4 hours. There was no apparent association between either of these pharmacokinetic variables and doxorubicin dose level; however, a linear relationship was found between core body temperature and log of plasma doxorubicin clearance (Fig. 3).

Tumor microdialysate was collected from five dogs. Tumor microdialysate concentrations were readily detectable within 30 minutes of drug infusion (representative data shown in Fig. 1),

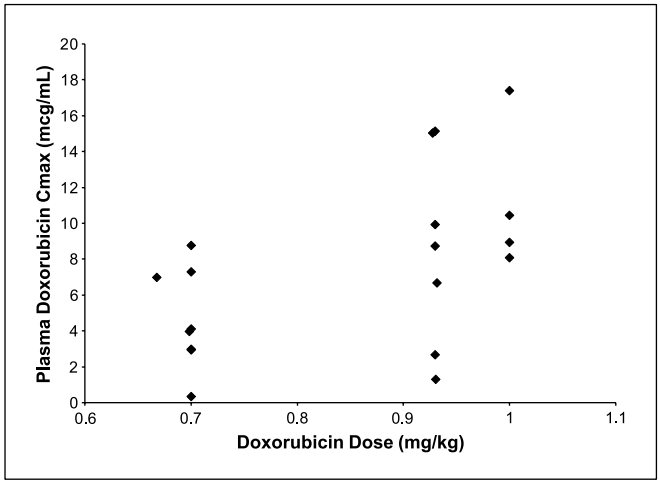

Fig. 2. Scatter plot showing the maximal observed doxorubicin plasma concentrations in each dog as a function of LTSL-doxorubicin dose level.

Table 2. Plasma pharmacokinetic variables

Patient ID	Dose level (mg/kg)	C_{max} (μ/mL)	AUC (mg/L h)	Clearance (L/h/kg)	$t_{1/2}$ (h)
92932	0.67	7.00	5.858	0.1140	1.751
89515	0.70	2.98	2.095	0.3341	1.480
90104	0.70	3.96	4.296	0.1626	0.994
89695	0.70	8.76	8.026	0.0872	1.221
168958	0.70	4.11	2.372	0.2951	0.196
98551	0.70	7.31	4.730	0.1480	0.495
189011	0.70	0.34	0.187	3.7513	0.331
99190	0.93	1.29	0.589	1.5813	0.128
190466	0.93	9.92	10.838	0.0858	0.399
70377	0.93	2.68	2.185	0.4256	0.387
97567	0.93	6.66	3.253	0.2864	0.846
194734	0.93	8.73	8.811	0.1055	0.505
130039	0.93	15.13	9.199	0.1011	NA
196219	0.93	15.03	29.613	0.0313	6.208
107747	1.00	10.46	15.404	0.0649	6.381
109046	1.00	17.40	151.529	0.0066	3.350
204641	1.00	8.93	12.585	0.0794	1.221
205112	1.00	8.08	5.625	0.1778	0.773
Mean (SD)				0.4355 (0.8998)	1.568 (1.939)

Abbreviation: NA, not available.

varied less within versus between patients (data not shown), and were linearly associated with log of maximal doxorubicin plasma concentrations (Fig. 4). The doxorubicin concentration was evaluated in 11 tumor biopsies from the 0.93 mg/kg dose (multiple samples from three patients) with an average value of 3.29 ± 1.83 ng/mg and nine samples in the 1.0 mg/kg dose (multiple samples from three dogs) with an average concentration of 9.12 ± 6.17 ng/mg.

Tumor response. Tumor response was not a primary end point of this study, and these tumors were typical of patients in a phase I trial: highly variable in clinical presentation. Differences in clinical presentation and histology make drawing broad conclusions regarding response rate inappropriate. The volume of the tumors at the start of treatment ranged from 3 to 1,747 cm^3 with a mean ± SD of 246 ± 380 cm^3 and a median of 91 cm^3. In the 20 patients that received at least two doses of LTSL-doxorubicin, the responses are as follows: two dogs (10%) had progressive disease (≥25% increase in tumor volume); 12 dogs (60%) had stable disease (<25% increase in tumor volume and <50% decrease in tumor volume); and six dogs (30%) had a partial response (>50% and <100% decrease in tumor volume). The solitary pulmonary lesion visualized in one dog could not be identified on radiographs posttreatment. A number of these tumors were too large to be completely heated with our technology. This may have led to a reduced response rate. Because this was a phase I trial, large tumors were acceptable, as tumor response was a secondary end point.

Discussion

LTSL-doxorubicin was well tolerated by most canine patients. The MTD of 0.93 mg/kg reported here is slightly lower than the MTD of free doxorubicin or Doxil in dogs and may be related in part to the toxicities resulting from the hypersensitivity reactions seen with the LTSL infusion. The species sensitivity of dogs to liposomally encapsulated doxorubicin can result in a significant drop in arterial pressure with resulting renal damage and necessitates appropriate premedication. This effect has not been previously documented, although published reports suggest it has been seen with other liposomal formulations as well (11). The administration of the LTSL to anesthetized and instrumented dogs in this study made recognition of this toxicity more likely. Although "complement activation–related pseudoallergy" (with acute symptoms similar to those seen in these dogs) has been reported in people receiving liposomally encapsulated drugs, the clinical incidence is typically <10% and

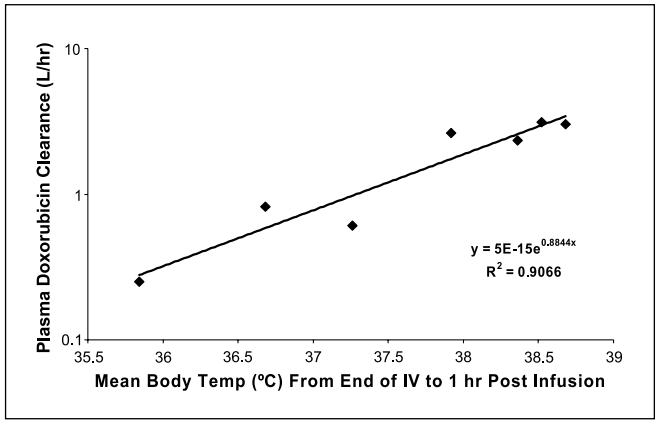

Fig. 3. Relationship between mean body temperature and LTSL-doxorubicin systemic clearance rate. The mean body temperature was calculated from the end of the infusion until the end of the heat application (1 hour) based on the deep rectal temperatures and graphed against the calculated rate of systemic drug clearance.

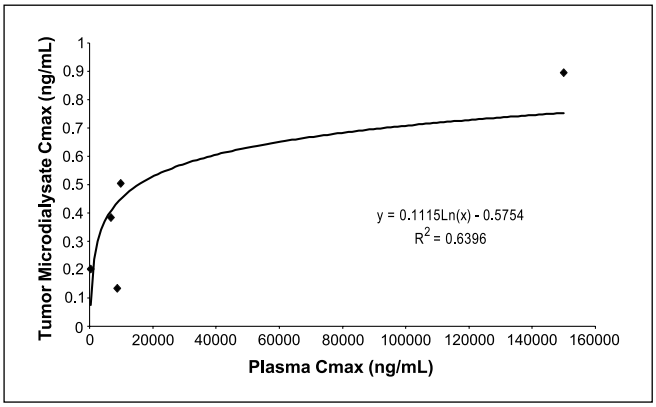

Fig. 4. Relationship between ln of the observed maximal plasma doxorubicin and tumor microdialysate doxorubicin concentrations following treatment with LTSL-doxorubicin and local hyperthermia. For the five dogs for whom microdialysate data were available.

can be controlled with appropriate pretreatment and a decreased infusion rate (20, 21).

The pharmacokinetics of the LTSL-encapsulated doxorubicin more closely resemble those of free drug than traditional liposome encapsulated drug, a finding consistent with the rapid release of drug within the tumor vasculature induced by local heating (9). Acute toxicities seen (myelosuppression) are also similar to those experienced with free drug rather than the cutaneous toxicity typically reported with liposome-encapsulated doxorubicin.

Doxorubicin clearance was decreased ~17-fold with the LTSL formulation when compared with the reported clearance of free doxorubicin (0.4355 L/h/kg for LTSL versus 7.362 L/h/kg for free drug; ref. 22); however, it was highly variable between patients. The decreased clearance results in a higher AUC seen in the patients treated with the LTSL-doxorubicin. For comparison, in four dogs treated at the 1.0 mg/kg dose level, the average AUC was 46.3 ± 60.9 mg/L hours. In eight dogs that received 1.0 mg/kg free doxorubicin i.v. over 10 minutes, the average AUC was 0.159± 0.026 mg/L hours (22).

Tumor drug levels were variable and were not significantly associated with C_{max} or AUC. There was a significant association between drug dose and tumor drug level ($P < 0.05$). One explanation for the degree of variability may be differences in vascular density between tumors. More highly vascularized tumors may allow more efficient drug delivery. Likewise, most drug levels were measured on a single biopsy sample; thus, variability in drug concentration within the tumor may have an effect on these results. Some tumors were large, and a large tissue volume was heated, resulting in a measurable elevation of whole body temperature. An elevation in core temperature would cause systemic drug release from the liposomes and result in lower tumor drug concentrations consistent with those obtained with the administration of free doxorubicin. Despite these potentially complicating factors, the i.t. drug levels achieved at the 1.0 mg/kg dose level were ~10-fold higher than what was achieved with free doxorubicin and hyperthermia in a mouse model (23).

Although not a primary end point, the response rate of 30% was slightly higher than anticipated, particularly considering that some tumors were incompletely heated due to their large size or location, and the dose of LTSL-encapsulated doxorubicin was suboptimal in many patients. In a phase II trial of doxorubicin in dogs with a variety of tumor types, the reported response rate of sarcomas was 22% and that of carcinomas was 26% (12). The response rate seen in our patients, and the 60% of patients with stable disease, is encouraging, although the effect of the local hyperthermia treatment can not be independently evaluated.

In conclusion, the MTD of LTSL-encapsulated doxorubicin in dogs with spontaneous tumors is 0.93 mg/kg when given i.v. over 30 minutes concurrently with hyperthermia. The presence of a species specific hypersensitivity-like reaction requires appropriate pretreatment and monitoring in this population and could possibly be seen in human applications of this drug formulation as well. The toxicity profile, aside from the acute systemic histamine release, was acceptable, and the encouraging tumor responses support further evaluation of LTSL-encapsulated doxorubicin for the treatment of appropriate cancers in people.

Acknowledgments

We thank Drs. Cary Bassett and Nancy Gustafson, Michael Lora-Michaels, Blaine Mischen, Cheryl Morgan, and Frank Conway for their technical and analytic assistance with this study.

References

1. Chidiac T, Budd GT, Pelley R, et al. Phase II trial of liposomal doxorubicin (Doxil) in advanced soft tissue sarcomas. Invest New Drugs 2000;18:253–9.
2. Ellerhorst JA, Bedikian A, Ring S, Buzaid AC, Eton O, Legha SS. Phase II trial of Doxil for patients with metastatic melanoma refractory to frontline therapy. Oncol Rep 1997;6:1097–9.
3. Garcia AA, Kempf RA, Rogers M, Muggia FM. A phase II study of Doxil (liposomal doxorubicin): lack of activity in poor prognosis soft tissue sarcomas. Ann Oncol 1998;9:1131–3.
4. Halford S, Yip D, Karapetis CS, et al. A phase II study evaluating the tolerability and efficacy of CAELYX (liposomal doxorubicin, Doxil) in the treatment of unresectable pancreatic carcinoma. Ann Oncol 2001; 12:1399–402.
5. Mills JK, Needham D. The materials engineering of temperature-sensitive liposomes. Methods Enzymol 2004;387:82–113.
6. Yatvin MB, Weinstein JN, Dennis WH, Blumenthal R. Design of liposomes for enhanced local release of drugs by hyperthermia. Science 1978;202:1290–3.
7. Gaber MH, Hong K, Huang SK, Papahadjopoulos D. Thermosensitive sterically stabilized liposomes: formulation and *in vitro* studies on mechanism of doxorubicin release by bovine serum and human plasma. Pharm Res 1995;12:1407–16.
8. Samulski TV, Fessenden P, Lee ER, Kapp DS, Tanabe E, McEuen A. Spiral microstrip hyperthermia applicators: technical design and clinical performance. Int J Radiat Oncol Biol Phys 1990;18:233–42.
9. Needham D, Anyarambhatla G, Kong G, Dewhirst MW. A new temperature-sensitive liposome for use with mild hyperthermia: characterization and testing in a human tumor xenograft model. Cancer Res 2000;60:1197–201.
10. Eisenhauer EA, O'Dwyer PJ, Christian M, Humphrey JS. Phase I clinical trial design in cancer drug development. J Clin Oncol 2000;18:684–92.
11. Vail DM, Kravis LD, Cooley AJ, Chun R, MacEwen EG. Preclinical trial of doxorubicin entrapped in sterically stabilized liposomes in dogs with spontaneously arising malignant tumors. Cancer Chemother Pharmacol 1997;39:410–6.
12. Ogilvie GK, Reynolds HA, Richardson RC, et al. Phase II evaluation of doxorubicin for treatment of various canine neoplasms. J Am Vet Med Assoc 1989;195:1580–3.
13. Hope MJ, Bally MB, Webb G, Cullis PR. Production of large unilamellar vesicles by a rapid extrusion procedure. Characterization of size distribution, trapped volume and ability to maintain a membrane potential. Biochim Biophys Acta 1985; 812:55–65.
14. Mayer LD, Bally MB, Hope MJ, Cullis PR. Uptake of antineoplastic agents into large unilamellar vesicles in response to a membrane potential. Biochim Biophys Acta 1985;816:294–302.
15. Dewhirst MW, Phillips TL, Samulski TV, et al. RTOG quality assurance guidelines for clinical trials using

hyperthermia. Int J Radiat Oncol Biol Phys 1990;18:1249–59.

16. Maguire PD, Samulski TV, Prosnitz LR, et al. A phase II trial testing the thermal dose parameter CEM43 degrees T90 as a predictor of response in soft tissue sarcomas treated with pre-operative thermoradiotherapy. Int J Hyperthermia 2001;17:283–90.

17. Vujaskovic Z, Poulson JM, Gaskin AA, et al. Temperature-dependent changes in physiologic parameters of spontaneous canine soft tissue sarcomas after combined radiotherapy and hyperthermia treatment. Int J Radiat Oncol Biol Phys 2000;46:179–85.

18. Ettinger SN, Poellmann CC, Wisniewski NA, et al. Urea as a recovery marker for quantitative assessment of tumor interstitial solutes with microdialysis. Cancer Res 2001;61:7964–70.

19. Secord AA, Jones EL, Hahn CA, et al. Phase I/II trial of intravenous Doxil and whole abdomen hyperthermia in patients with refractory ovarian cancer. Int J Hyperthermia 2005;21:333–47.

20. Chanan-Khan A, Szebeni J, Savay S, et al. Complement activation following first exposure to pegylated liposomal doxorubicin (Doxil): possible role in hypersensitivity reactions. Ann Oncol 2003;14:1430–7.

21. Szebeni J. Complement activation-related pseudoallergy caused by liposomes, micellar carriers of intravenous drugs, and radiocontrast agents. Crit Rev Ther Drug Carrier Syst 2001;18:567–606.

22. Arrington KA, Legendre AM, Tabeling GS, Frazier DL. Comparison of body surface area-based and weight-based dosage protocols for doxorubicin administration in dogs. Am J Vet Res 1994;55:1587–92.

23. Kong G, Anyarambhatla G, Petros WP, et al. Efficacy of liposomes and hyperthermia in a human tumor xenograft model: importance of triggered drug release. Cancer Res 2000;60:6950–7.