Hyperthermia Increases Accumulation of Technetium-99m-labeled Liposomes in Feline Sarcomas

Mark L. Matteucci, Gopal Anyarambhatla, Gary Rosner, Chieko Azuma, Paul E. Fisher, Mark W. Dewhirst, David Needham, and Donald E. Thrall


ABSTRACT

The effect of hyperthermia on the accumulation of technetium-99m-labeled liposomes was studied in feline sarcomas. Each cat received two separate injections of liposomes. The first was used to quantify the amount of technetium-99m-labeled liposomes within the tumor under normothermic conditions. The second injection was made at the beginning of a 60-min hyperthermia procedure. Planar scintigraphy was used to measure the activity of technetium-99m-labeled liposomes within the tumor at predetermined times up to 18 h after injection. Regions of interest were drawn for the tumor, lungs, liver, kidney, and aorta. Counts in the regions of interest were decay corrected. Counts/pixel in the tumor under normothermic and hyperthermic conditions were normalized to aorta counts/pixel. A total of 16 cats were eligible for the study. In two of the 16 cats, incomplete count data precluded analysis. In the remaining 14 cats, hyperthermia resulted in a significant increase in liposome accumulation in the tumor (P = 0.001). Tumor volume ranged from 1.2 to 236.2 cm³, and thermal dose ranged from 2.0 to 243.3 CEM43CT90 (equivalent time that the 10th percentile temperature was equal to 43°C). There was not a relationship between either tumor volume or hyperthermia dose on the magnitude of increased liposome accumulation, suggesting that this method has application across a range of tumor volumes and degrees of heatability.

INTRODUCTION

Systemic administration of drugs to treat solid tumors is nonspecific. The amount of drug reaching the target tumor cell will be influenced by the pharmacokinetic behavior of the drug, tumor perfusion, drug transport into the extracellular space, and microenvironmental characteristics, such as the extracellular/intracellular pH gradient (1–3). Classic methods used to increase the therapeutic effects of systemic chemotherapy include modification of the schedule or dose intensity of drug administration (4), use of drug combinations (5), and intracavitary or intraarterial routes of administration (6, 7). Although systemic toxicity and drug resistance remain as impediments to improving tumor response through administration of drugs, the strategies mentioned above have resulted in improvements in response in some instances. However, there still is opportunity for gain by identifying ways to selectively increase intravascular, intratissue, and intracellular drug concentration. Encapsulation of a drug within liposomes represents one way the intravascular, intratissue, and intracellular drug concentration could be increased. In this study, we tested whether hyperthermia could be used to augment delivery of liposomes to tumors, specifically to increase intratissue concentration of the liposome. If hyperthermia was capable of enhancing liposome accumulation, it is expected that if the liposome contained a drug that the drug will eventually leak out and increase the intracellular concentration of drug in the tumor cells themselves.

Liposomes are small vesicles made of synthetic or natural lipids. Liposome size can be controlled, depending on their intended use, such as drug delivery or imaging (8). Individual vesicles can be manufactured to contain substances of varying water or oil solubility, including drugs. The inherent leakiness of tumor blood vessels has been shown to result in some degree of selectivity of liposomal delivery. The basis for intratumoral liposome accumulation is enlarged endothelial pores. Pore size has been quantified in rodent tumors, and they are large enough to allow 100–200-nm-diameter liposomes to extravasate and accumulate in the tumor (9, 10). These pores are heterogeneously distributed throughout the tumor vasculature and provide a heterogeneous route for liposome accumulation into the tumor extravascular tissue. Once in the tumor interstitial space, liposomes, because of their relatively large size, tend to reside preferentially in the perivascular space just outside the blood vessels and do not become homogeneously distributed throughout the tumor.

When injected i.v., conventional liposomes are quickly coated with plasma opsonins and lipoproteins. Subsequently, reticuloendothelial cells phagocytize and remove these liposomes from the circulation, resulting in a circulation half-life of ~30 min and relatively low numbers of liposomes in the tumor. By modifying the surface of liposomes using PEG, liposome

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2 To whom requests for reprints should be addressed, at College of Veterinary Medicine, North Carolina State University, 4700 Hillsborough St., Raleigh, NC 27606. Phone: (919) 513-6292; Fax: (919) 513-6578; E-mail: don_thrall@ncsu.edu.

3 The abbreviations used are: PEG, polyethylene glycol; HMPAO, hexamethyl propyleneamine oxime; GSH, reduced glutathione; ROI,
circulation half-life can be extended (11, 12). PEG-coated liposomes evade phagocytosis by the reticuloendothelial system (13). The extended circulation half-life is attributable to attraction of water to the liposome surface, which forms a repulsive steric barrier (14–16) for protein molecules that would normally bind to and label the liposome as foreign material destined for phagocytosis. The presence of the hydrophilic PEG coating, therefore, results in enhanced stability by inhibiting interactions with plasma proteins such as opsonins and lipoproteins (17, 18). Additionally, it has been shown that PEG coating increases vascular permeability, leading to a severalfold greater accumulation of drug-containing liposomes in diseased tissue compared with normal tissue (9).

Hyperthermia has been used in both humans and animals for multimodality treatment of cancer. Hyperthermia may sensitize tumor cells to radiation and increase cytotoxicity based on its ability to kill cells without regard for some factors that render cells radiation resistant, such as cell cycle phase and oxygenation status. Local hyperthermia in combination with radiation has been shown to be superior to radiation alone in studies both in animals (19–21) and humans (22–25). Effects of hyperthermia leading to improved tumor response include direct cytotoxicity and alteration of the tumor microenvironment (26). Clinical use of hyperthermia has typically been based on one of these principles. However, there are other effects of hyperthermia that provide an opportunity for achieving improved tumor response. For example, hyperthermia has been shown to increase both tumor blood flow and vascular permeability (26).

It has been shown in a variety of murine models that hyperthermia can be used to further enhance liposomal drug delivery to tumors. In a published review of the combination of hyperthermia and liposomes in rodents, 15 studies were identified where hyperthermia and liposomes resulted in increased intratumoral drug concentration in comparison to hyperthermia plus free drug or liposomal drug alone (27). Seventeen additional studies were found where hyperthermia and liposomes resulted in enhanced tumor growth delay in comparison to hyperthermia plus free drug or liposomes alone (27). Clearly, these results suggest there may be clinical utility in the combination of hyperthermia and liposomally encapsulated drug for treatment of solid tumors. It is important that these results obtained from studies of implanted tumors in rodents be verified in spontaneous, solid tumors. Also, the magnitude of the hyperthermic effect on increased liposome accumulation in spontaneous tumors will likely parallel results to be expected in animals (19–21) and humans (22–25). Additional studies will be required to determine the extent to which results from rodent trials can be expected in spontaneous, solid tumors. Also, the magnitude of the hyperthermic effect on increased liposome accumulation in spontaneous tumors will likely parallel results to be expected in humans more closely than results from rodent trials. In this paper, we describe the effect of hyperthermia on liposome accumulation in feline fibrosarcomas by quantifying the uptake of technetium-99m-labeled liposomes in the tumor under normothermic and hyperthermic conditions.

MATERIALS AND METHODS

Animal Model. Privately owned cats bearing a soft tissue sarcoma were studied. Cats were presented for treatment at the College of Veterinary Medicine, North Carolina State University. This study was approved by the University’s Institutional Animal Care and Use Committee. Most soft tissue sarcomas in adult cats are vaccine-associated sarcomas. Vaccine-associated feline sarcomas are thought to arise from malignant transformation of chronic inflammation, induced by some fraction of the vaccine (28, 29). Regardless of the fact that these tumors are vaccine associated, they are extremely refractory to therapy. Once established, they are not characterized by spontaneous regression, and there is a significant incidence of distant metastasis and recurrence after incomplete excision. They are aggressive malignant tumors. Obtaining permanent local control using surgery is generally not possible without amputation of the affected part. Radiation and surgery have been used for therapy with some success (30). For a cat to be included in this study, the tumor had to be in a location that permitted thermometry and heating. Tumors with bone invasion were ineligible because of the inherent difficulty in adequately heating such tumors. Computed tomographic images of all tumors were acquired to ascertain tumors did not involve bone. Tumor volume was estimated by multiplying the product of three orthogonal diameters by π/6.

Radiopharmaceuticals. Hydrogenated soy sn-glycero-3-phosphocholine, cholesterol and 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-poly(ethylene glycol) 2000 were purchased from Avanti Polar Lipids. A HMPAO kit (Ceretec) was purchased from Amersham, Inc. (Arlington Heights, IL). Dulbecco’s PBS and GSH were from Sigma Chemical Co. Sephadex G-50 was purchased from Pharmacia. Sodium pertechnetate was obtained from the Radiopharmacy at Duke University Medical Center. All of the other chemicals were of reagent grade and were used without further purification.

Liposome Preparation. Lipid vesicles were prepared by the lipid film hydration method described previously (31). Briefly, required amounts of the phospholipids sn-glycero-3-phosphocholine, cholesterol, and 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-poly(ethylene glycol) 2000 (75:50:3 molar ratio) were dissolved in chloroform, and the solvent was evaporated under vacuum using a Rotavapor R-124 (Rotavapor, Buchi, Switzerland) to form a thin film on the inner walls of the round-bottomed glass container. The film was dried with a stream of nitrogen gas to remove traces of organic solvent and vacuum dried overnight. Hydration of the lipid film was carried out using 200 μM GSH in Dulbecco’s PBS (pH 7.40) at 58–60°C, a temperature a few degrees above the transition temperature of the main lipid used. During the hydration procedure, a small magnetic bar was used to stir and facilitate suspension of the lipid film into the hydrating medium. The multilamellar vesicles thus formed were subjected to extrusion using a thermostable extruder (Lexip Biomembranes, Vancouver, British Columbia, Canada) at 58°C and ~300–400 psi pressure through nanopore filters of 100-nm pore size (31). Unentrapped GSH was removed from the liposome suspension by passing it through a Sephadex G-50 column. The size of the resultant large unilamellar liposomes was determined by Photon Correlation

region(s) of interest; T50, median temperature; T90, 10th percentile temperature; CEM43CT50, equivalent time that median temperature was equal to 43°C; CEM43CT90, equivalent time that the 10th percentile temperature was equal to 43°C.
Spectroscopy (N4 Plus; Coulter Corp., Miami, FL) to be in the range of 180–200 nm.

**Labeling of Preformed Liposomes using Technetium 99m.** Radiolabeling of liposomes using $^{99m}$Tc was carried out as described previously (32). Briefly, an HMPAO kit (Ceretec) containing 0.5 mg of HMPAO and 4.0 μg of SnCl$_2$ was reconstituted with 20 mCi of sodium pertechnetate ($^{99m}$TcO$_4^-$) in 0.9% NaCl solution and incubated at room temperature for 5 min to form the $^{99m}$Tc-HMPAO complex, which was then mixed with preformed liposomes and incubated for 30 min at room temperature with intermittent vortexing. Free $^{99m}$Tc label was removed from $^{99m}$Tc-liposomes by passing the suspension through a Sephadex G-50 column. Labeling efficiency was determined by the following equation:

$$E(\%) = \frac{A_{\text{Liposomes}}}{A_{\text{Total}}} \times 100$$

where $E$ is labeling efficiency, $A_{\text{Liposomes}}$ is activity associated with the vesicle suspension, and $A_{\text{Total}}$ is total activity. The labeling efficiency was in the range of 90–92%. We did not measure the amount of spontaneous technetium dissociation from the liposomes. However, liposomes labeled with the $^{99m}$Tc-HMPAO method have excellent in vivo stability in serum (6% dissociation at 4 h and only 15% dissociation after 90 h at 37°C; Ref. 32). Additionally, any dissociated technetium would be taken into account by correcting for activity in the aorta ROI (see below).

**Normothermic Study.** Each cat was anesthetized to facilitate placement of i.v. catheters, intratumoral thermometry catheters, and computed tomography. Anesthesia was induced with isoflurane and oxygen via a facemask. After anesthesia induction, an endotracheal tube was inserted, and anesthesia was maintained with 2.5% isoflurane and 1 l/min oxygen. Each cat had a cephalic vein catheter dedicated for liposome injection. Thermometry probes were inserted in each tumor. The use of thermometry probes for normothermic study functioned as a control for any possible effect of probe placement on liposome accumulation. For each tumor, at least two thermometry catheters were inserted under sterile conditions. Thermometry catheter positions were evaluated with computed tomography. After the i.v. and thermometry catheters were inserted and the computed tomography images were acquired, the cat was placed on the collimator of a gamma camera, and 3–5 mCi (111–185 MBq) of $^{99m}$Tc-liposomes were injected i.v. via the cephalic catheter. One-min scintigram images were acquired at the time of injection and at 5, 15, and 30 min after liposome injection. The cats were allowed to recover from anesthesia after the 30-min postinjection image acquisition. At this same time, the cephalic catheter was removed and, along with the injection syringe, was placed in a dose calibrator to quantify the net injected liposome dose, expressed in mCi. The thermometry probes were removed from the tumor. Subsequent images were acquired at approximately 90, 120, 150, 180, 210, 240, 270, 300, 330, 360, 480, and 1080 min after liposome injection, with the cat awake and held on the collimator in a consistent position.

**Hyperthermic Study.** Forty-eight h (eight half-lives) after the normothermic study, a time sufficient to allow for $^{99m}$Tc decay resulting in no visible detection of radioactivity with the gamma camera, the hyperthermic study was performed on the same animal. Anesthesia was the same as in the normothermic study. Thermometry probes were placed in the tumor, and computed tomography was performed to evaluate probe positioning. A cephalic catheter was inserted and used only for liposome injection.

Local hyperthermia was delivered with microwaves using a stationary (407 MHz) or scanning spiral (433 MHz) applicator, depending on the tumor size and location. Deionized water was used as a coupling medium. Surface cooling was provided with deionized water maintained at a temperature preventing the skin from reaching 43°C. Upper limits of 43°C and 48°C were placed on normal tissue and tumor temperature, respectively. The microwave power level was adjusted to maintain intratumoral and skin temperatures within this range. Skin temperatures were also monitored. The hyperthermia treatment was 60 min in duration, beginning with the registration of a 42°C temperature at any point in the tumor. The thermometry goals were to sample the temperature profile across the major axis of the tumor, through the tumor/normal tissue interface, and at a deep margin of the tumor. Normal tissue thermometry was also performed with particular attention to areas of scar tissue. Information from the computed tomography images was used to determine positions along the catheter as measuring tumor or normal tissue temperature. Fluoroptic temperature measuring devices were used. Thermometers were calibrated before each treatment, and accuracy was within 0.2°C. A photograph and sketch of the tumor and thermometry catheters at each hyperthermia procedure allowed for registration of measured temperatures with the spatial characteristics of the tumor and normal tissue.

Manual or automated thermal mapping of temperature along thermometry catheter tracks was performed during treatment by withdrawal of thermometers in their catheters. Temperature was measured at 0.5-cm steps in thermometry catheters. At least five positions were measured along each catheter track. When using the manual technique, thermal mapping was done whenever power adjustments were made, the position of the applicator was adjusted, or when the temperature at points of static measurement was noted to be changing.

The start of the hyperthermic treatment time was defined as any intratumoral temperature $\geq 42$°C. At that time, as in the normothermic study, 3–5 mCi (111–185 MBq) of $^{99m}$Tc-liposomes were injected into the dedicated cephalic catheter. At the end of the hyperthermia treatment, the cat was placed on the gamma camera collimator in the same position as the normothermic study. For heated tumors, the initial image was acquired approximately 1 h after injection of liposomes. In fact, the exact time of this image after liposome injection ranged from 60 to 70 min; variability was introduced by the need to transport the cat from the hyperthermia room to the scintigraphy lab. Regardless, this initial image was termed the 60-min image. The cats were allowed to recover from anesthesia after the first postinjection image acquisition. At this same time, the cephalic catheter was removed and, along with the injection syringe, was placed in a dose calibrator to determine the net injected liposome dose, expressed in mCi. The thermometry probes were also removed. Subsequent images were acquired at approximately 90, 120, 150, 180, 210, 240, 270, 300, 330, 360, 480, and 1080 min after
liposome injection, with the cat awake and held on the collimator in a consistent position.

The method of reducing time-temperature data has been described in detail (33). Briefly, from the temperatures measured in thermometry catheters, an array of temperatures during each 1-min time interval was obtained using linear interpolation between successive temperature measurements at a given site. From these data, the minimum temperature, maximum temperature, T50, T90, CEM43T50, and CEM43CT90 were calculated.

**Scintigaphy.** Planar scintigraphy was used to quantify the activity of 99mTc-labeled liposomes in the tumor at predetermined time points up to 18 h after injection. Whole-body scintigrams were acquired using a gamma camera equipped with a low energy all-purpose collimator. The camera was interfaced to a computer with nuclear imaging software. Image acquisitions were each for 1 min using a 256 × 256 matrix. The cat was positioned on the camera face either in dorsal or lateral recumbency, depending on the location of the tumor.

ROI were drawn for the tumor, lungs, liver, kidney, and aorta at each time point. The aorta counts served as a measure of both circulating and background liposomes. Any free 99mTc would also be accounted for in the aorta counts. Counts in the ROI were decay corrected. Counts/pixel in the tumor under normothermic and hyperthermic conditions were normalized to the aorta counts/pixel to determine the effect of hyperthermia on accumulation of liposomes. Counts/pixel in the lung, liver, and kidney under normothermic and hyperthermic conditions were also normalized to the aorta counts/pixel to assess for any effect of hyperthermia on liposome uptake in these normal tissues.

**Statistical Analysis.** We plotted aorta-normalized tumor counts/pixel as a function of time for each cat, and plots for normothermic and hyperthermic conditions were visually compared. We used nonparametric statistical methods to compare normothermic and hyperthermic results. Using the ROI of the tumor and organs, the effect of hyperthermia was tested using the Wilcoxon signed rank test. The analysis was performed by calculating the average of the tumor counts/pixel normalized to the aorta counts/pixel. Averages were calculated from 200 min to last acquisition and from 400 min to last acquisition. This was done for the normothermic and hyperthermic study. The time-averaged method was used because not all scintigraphic acquisitions were performed at exactly the same time after liposome injection across cats or within cats. This variability resulted from the fact that liposome injections and hyperthermia were performed at various times during the day, making it difficult to follow an identical sampling schedule during evening hours. The liver, lung, and kidney average counts were similarly analyzed. The significance of correlations was evaluated using the Spearman rank correlation coefficient nonparametric test.

**RESULTS**

Sixteen cats were eligible for the study. In one cat, liposomes were given by mistake at the end of heating rather than at the start, and in another cat, the tumor was too small (<0.5 cm³) to image satisfactorily. This left 14 cats with data suitable for analysis of concurrent heat plus liposomes. Tumor volume ranged from 1.2 to 236.2 cm³ (Table 1). Variation in thermal dose was present between cats (Table 1); no cat experienced thermal toxicity. In all cats, the tumor was visible in scintigraphic images, and accumulation appeared qualitatively greater after hyperthermia and continued to increase after heating was discontinued (Fig. 1). Quantitatively, hyperthermia resulted in a significant increase in liposome accumulation in the tumor (P = 0.001). This was true for time-averaged counts/pixel beginning 200 min and 400 min after the start of the experiment. In most cats, the time-averaged magnitude of increased liposome accumulation at times >200 min after injection caused by the 60-min heating ranged from two to four times (Fig. 2). Instantaneously, greater differences between normothermic and hyperthermic accumulation were noted in all cats (Fig. 3).

There was no significant difference in liposome accumulation in the liver, lung, or kidney between hyperthermic versus normothermic conditions. In 3 or 4 of the 14 cats, depending on the organ being considered, liposome accumulation was increased in lung, liver, or kidney after hyperthermia. However, in the remaining cats, the hyperthermia:normothermia ratio was typically <1.0 in comparison to the ratio of 2:4 in the tumor (Fig. 3), and based on time-averaged ratios, there was no difference. Finding a ratio of ~1.0 was expected, because it has been shown by others that normal vessels are much less permeable to these relatively large liposomes and also because these organs were not heated. There was not a relationship between hyperthermia dose on the magnitude of increased accumulation, regardless of the thermal descriptor used. Finally, there was no relationship between tumor volume and the effect of hyperthermia on liposome accumulation.

**DISCUSSION**

The major finding from this study is that, like the rat model studies, hyperthermia significantly increased the accumulation of liposomes, but now in a spontaneous, solid tumor. Our results confirm the benefits of using hyperthermia as a method to target delivery of liposomes to tumors and are consistent with results found in other experimental tumor models (27). Clinical evaluation of chemotherapy-containingliposomes combined with hyperthermia is a logical extension of this concept.

The rise in intratumoral counts over time ranging from 200 to 1000 min after injection indicates that accumulation of liposomes out of the blood and into the tumor continued even after conclusion of the 60-min hyperthermia procedure. We believe there are two reasons for increased liposome accumulation after heating:

(a) Effects of heat on tumor vasculature have been well documented. Hyperthermia causes an increased size to the endothelial junctions, which result in increased vascular permeability (34, 35). In prior studies (10), it was determined that the

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**Table 1** Volume and thermal dose parameters

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<th>Median</th>
<th>Range</th>
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<tr>
<td>Tumor volume (ccm)</td>
<td>20.7</td>
<td>1.2–236.2</td>
</tr>
<tr>
<td>CEM43T50 (min)</td>
<td>249</td>
<td>48–605</td>
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<tr>
<td>CEM43T90 (min)</td>
<td>20</td>
<td>2–243</td>
</tr>
<tr>
<td>T50 (C)</td>
<td>44.6</td>
<td>42.9–46.1</td>
</tr>
<tr>
<td>T90 (C)</td>
<td>41.6</td>
<td>40.1–44.6</td>
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endothelial pore sizes in tumor vessels are between 400 and 600 nm. Because the aorta-normalized tumor counts/pixel in the tumor continued to increase after hyperthermia, the vascular permeability changes caused by heating likely persisted, allowing continued liposome accumulation from the vasculature into the tumor interstitial space, as reported previously (36).

(b) The potential for sterically stabilized liposomes to remain in the circulation and evade the reticuloendothelial system may allow enhanced liposome accumulation. However, reticuloendothelial uptake was observed in cats in this study, because there was liposome accumulation in tissues with a high phagocytic activity, such as liver and spleen. Additionally, in cats there is a tendency for (nonpegylated) colloidal-sized particles to accumulate in the lungs. This is known based on the accumulation of sulfur-colloid in the lungs of cats undergoing hepatic scintigraphy (37) and is thought to be attributable to phagocytosis by intravascular macrophages (38). In other animal models, the majority of colloidal sized particles (80–90%) are removed by the Kupffer cells of the liver. It is possible in this study that the removal of liposomes by the lungs decreased their accumulation into the tumor in comparison with what might be observed in other species. Regardless of the long circulation time of these liposomes, under normothermic conditions intratumoral liposome concentration was essentially unchanged in comparison with that present in the aorta (Fig. 3). Thus, without hyperthermia, there may be little reason to expect
selective delivery of liposomes to a solid tumor. But, with use of hyperthermia, enhanced accumulation clearly occurred (Fig. 3).

We did not measure the thermal stability of the liposomes used in this study. However, we have no reason to suspect that thermal degradation was a major factor. In prior work by our group, there clearly was no release of doxorubicin from similar liposomes under hyperthermic conditions (39). Also, the pattern of increasing tumor radioactivity as a function of time after hyperthermia is not typical of free technetium because $^{99m}$Tc has no avidity for soft tissue sarcomas. Thus, the kinetics of increasing radioactivity are consistent with accumulation of liposomes into the tumor.

We injected the $^{99m}$Tc-labeled liposomes at the beginning of the hyperthermia treatment as in previous work investigating liposomes and hyperthermia (36, 38). Injection at the beginning of hyperthermia treatment may maximize accumulation because of the immediate changes in tumor vasculature induced by hyperthermia. The effect of delayed liposome administration after heating has been investigated in one of our laboratories (M. W. D.) using a murine FaDu human tumor xenograph model (27). The magnitude of liposome accumulation is similar up to 2 h after hyperthermia as simultaneous administration with hyperthermia. There is less liposome accumulation if administration is delayed to 4 h after hyperthermia. When administered 6 h after hyperthermia, the magnitude of accumulation is the same as in nonhyperthermic conditions. Therefore, it appears that because of the ability of the vascular endothelium to reseal (and become thermotolerant), the optimum time to administer liposomes is simultaneously or within 2 h of hyperthermia.

Thermal dose and tumor volume were carefully measured in this study, and we found no relationship between liposome accumulation and either of those two parameters. Clearly, there is some relationship between accumulation and temperature, i.e., there is some requisite temperature elevation needed for enhanced accumulation, and there is likely a maximum temperature above which accumulation will be impeded because of hyperthermic destruction of tumor microvasculature. However, in this study, the hyperthermia technique provided a window wherein accumulation was not related to temperature. This finding, and the lack of an effect of tumor volume on accumulation, increases the clinical utility of this method to a range of tumor volumes and heatability.

Finally, advances in liposome engineering have led to the
development of thermosensitive liposomes (40). These liposomes are mainly composed of a lipid (dipalmitoylphosphatidylcholine) that has a gel-to-liquid phase transition of 41.5–41.9°C. At this phase transition, it is known that the encapsulating membrane becomes leaky to the small drug molecules contained in the liposome. These liposomes then are designed to change from a gel-to-liquid phase at a specific temperature, with subsequent release of encapsulated drug. In vitro, it was found that hyperthermia enhanced the anti-tumor effects of thermosensitive liposome-encapsulated cisplatin in human osteosarcoma cells. Hyperthermia caused release of drug from the liposomes, resulting in greater intracellular accumulation of cisplatin (41). Similarly, in a study using a murine mammary adenocarcinoma, there was a significant increase in liposome accumulation and also a greater release of doxorubicin into the interstitium when encapsulated in thermosensitive liposomes (36). The temperature for these release studies was 42°C. In a new advanced design, in one of our laboratories (D. N.) a doxorubicin-containing, temperature-sensitive liposome has been engineered and optimized to rapidly (tens of seconds) release contents at hyperthermic temperatures (39–41°C) that are achievable in the clinic. This optimization of release rate and temperature of release is important because the uniform elevation of intratumoral temperature to a level expected to cause widespread release of content from a conventional (higher-temperature-releasing) thermosensitive liposome is a major problem to overcome. In tumor growth delay studies (39), doxorubicin encapsulated in nonthermally sensitive liposomes, traditional thermosensitive liposomes, and the new low-temperature sensitive liposomes was associated with significant growth delay when combined with hyperthermia. However, response with the low-temperature sensitive liposomes was much greater, and 17 of 20 mice had tumor control at 60 days. The prolonged tumor regrowth times seen with hyperthermia in combination with liposomes support the use of heat to enhance liposome accumulation in tumors for carriers that do not necessarily release drug in response to hyperthermia. However, the importance of increased liposomal delivery and the rapid release of drug by using a thermosensitive liposome that has a phase change in the temperature range achievable in large spontaneous tumors is apparent.

The positive effect of hyperthermia seen in this study justifies further trials. The ability to use liposomes to deliver drugs specifically to tumors has great potential in cancer therapy. It is reasonable that further investigation using hyperthermia for targeted delivery of liposomes and triggered drug release from thermosensitive liposomes may eventually result in increased clinical benefit.

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