Pulsed-High Intensity Focused Ultrasound and Low Temperature–Sensitive Liposomes for Enhanced Targeted Drug Delivery and Antitumor Effect

Sergio Dromi,[1] Victor Frenkel,[1] Alfred Luk,[1] Bryan Traughber,[1] Mary Angstadt,[1] Monica Bur,[1] Jason Poff,[1] Jianwu Xie,[1] Steven K. Libutti,[2] King C.P. Li,[1] and Bradford J. Wood[1]

Abstract

Purpose: To determine if pulsed-high intensity focused ultrasound (HIFU) could effectively serve as a source of hyperthermia with thermosensitive liposomes to enhance delivery and efficacy of doxorubicin in tumors.

Experimental Design: Comparisons *in vitro* and *in vivo* were carried out between non–thermosensitive liposomes (NTSL) and low temperature–sensitive liposomes (LTSL). Liposomes were incubated *in vitro* over a range of temperatures and durations, and the amount of doxorubicin released was measured. For *in vivo* experiments, liposomes and free doxorubicin were injected i.v. in mice followed by pulsed-HIFU exposures in s.c. murine adenocarcinoma tumors at 0 and 24 h after administration. Combinations of the exposures and drug formulations were evaluated for doxorubicin concentration and growth inhibition in the tumors.

Results: *In vitro* incubations simulating the pulsed-HIFU thermal dose (42°C for 2 min) triggered release of 50% of doxorubicin from the LTSLs; however, no detectable release from the NTSLs was observed. Similarly, *in vivo* experiments showed that pulsed-HIFU exposures combined with the LTSLs resulted in more rapid delivery of doxorubicin as well as significantly higher i.t. concentration when compared with LTSLs alone or NTSLs, with or without exposures. Combining the exposures with the LTSLs also significantly reduced tumor growth compared with all other groups.

Conclusions: Combining low-temperature heat-sensitive liposomes with noninvasive and nondestructive pulsed-HIFU exposures enhanced the delivery of doxorubicin and, consequently, its antitumor effects. This combination therapy could potentially produce viable clinical strategies for improved targeting and delivery of drugs for treatment of cancer and other diseases.

The dose of drug required to achieve clinically effective cytotoxicity in tumors often causes severe damage to actively propagating nonmalignant cells, resulting in a variety of undesirable side effects (1). Abnormal and heterogeneous distribution of inefficient vasculature (2), high interstitial fluid pressures (3), and fibrillar collagen in the extracellular matrix (4) are some of the barriers that further complicate effective and uniform drug delivery to tumors. Novel paradigms to overcome these barriers with new drug and device combinations may present fertile ground for continued research.

Employing drug delivery strategies, such as liposomal encapsulation, can optimize and enhance the delivery of different agents with lower systemic toxicity and better drug cell internalization compared with free drug (5). A smaller volume of distribution and prolonged clearance time may also be achieved by incorporating lipid-conjugated polyethylene glycol into the liposomal membrane. This polyethylene glycolylation provides a protective barrier against interactions with plasma proteins and the reticuloendothelial system, allowing for enhanced accumulation of the chemotherapeutic agent into tumors (6). Polyethylene glycolylated liposomes containing doxorubicin, or Doxil, have been used to treat Kaposi's sarcoma, refractory ovarian cancer, breast cancer, and other tumors (7).

Newer-generation liposomal chemistry has led to improved targeting and local drug delivery. This includes liposomes conjugated to antibodies, targeting ligands, or those that are pH or heat sensitive (8). The latter, also known as thermosensitive liposomes (TSL), release their payload in regions where local tissue temperatures are elevated (9), permitting their combination with an external source of hyperthermia, such as microwaves (10) or IR laser (11), for improved local drug delivery. TSLs have been combined with hyperthermia to show various potential therapeutic applications. In one study, a paramagnetic contrast agent was incorporated into TSLs with a relatively high

Authors' Affiliations: [1]Diagnostic Radiology Department, Clinical Center and [2]Tumor Angiogenesis Section, Surgery Branch, National Cancer Institute, NIH, Bethesda, Maryland

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Requests for reprints: Victor Frenkel, Molecular Imaging Lab, NIH Clinical Center, Room 1N306a, Building 10, 10 Center Drive, Bethesda, MD 20892. Phone: 301-402-5710; Fax: 301-496-9933; E-mail: vfrenkel@cc.nih.gov.

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transition temperature to monitor high-intensity focused ultrasound (HIFU)–induced ablation with magnetic resonance imaging (12). This combination therapy has also been used to enhance the delivery of various chemotherapeutic agents for improved antitumor effect, including doxorubicin (13), cisplatin (14), and methotrexate (15).

Compared with non-TSLs (NTSL) that remain stable and do not release drug in the physiologic temperature range, TSLs undergo a phase change when heated that renders the liposomes more permeable, releasing their payload (16). Traditional TSLs are triggered in the range of ~42°C to 45°C, releasing their drug over ~30 min, whereas low temperature–sensitive liposomes (LTSL) release their payload in matter of seconds in temperature ranges of 39°C to 40°C (13).

HIFU is presently being used to noninvasively ablate tumors, where relatively long, continuous exposures are employed, to produce the required high temperature elevations for thermal ablation and direct tumor destruction (17). If, however, shorter pulses are given in combination with relatively short duty cycles, temporal average intensities will be decreased. This will reduce the generation of heat and allow non-lethal temperature elevations (18), where interactions of ultrasound energy with exposed tissue will be primarily non-thermal. Such exposures, which generate transient temperature elevations of only 4°C to 5°C (19), have been used to noninvasively enhance local delivery of various macromolecules into different tissue types, improving their therapeutic effects (20).

In the present study, pulsed-HIFU exposures were combined with an experimental LTSL to enhance local delivery of doxorubicin into tumors, and these results were compared with that of a commercial NTSL. Initial experiments were first carried out *in vitro* to characterize heat-activated drug release and then followed with *in vivo* studies on both local drug deposition and therapeutic effects in a s.c. murine tumor model sensitive to doxorubicin.

Materials and Methods

Free and liposomal doxorubicin. Two different doxorubicin liposomal formulations were used: Doxil (Ortho Biotech Products) served as the NTSL, and ThermoDox (Celsion Corp.) served as the LTSL. The NTSLs are commercially available and were reconstituted in 5% dextrose according to the manufacturer's directions on the day of its use. The LTSLs were prepared on site, 1 day before use, according to the specific procedures provided by the manufacturer. Free doxorubicin (ALZA Pharmaceuticals, Inc.) was reconstituted with saline. All doxorubicin formulations were initially produced at a doxorubicin concentration of 2 mg/mL.

In vitro *drug release.* One milliliter (2 mg/mL) of liposomes (NTSL and LTSL) was placed in 2-mL RNA tubes suspended in a circulating water bath (Haake DC10-P5/U) at a range of temperatures (20-42°C) and durations (0-12 min) to study the release dynamics of doxorubicin in response to thermal dose. Four vials were used per group. Immediately after the incubations, three 100-μL samples were taken from each vial and transferred to a white, 96-well plate. The plate was read for 4 s per well with a LS-55 fluorimeter (Perkin-Elmer) at an excitation of 480 nm and emission of 590 nm, corresponding to doxorubicin (19). A standard curve was prepared for each plate that was read using known concentrations of doxorubicin, and the concentrations of experimental samples were consequently determined by interpolation.

Mice and tumors. All animal work was done according to an approved animal protocol and in strict compliance with NIH Clinical Center Animal Care and Use Committee guidelines and regulations. A murine mammary adenocarcinoma (JC) cell line was used for all studies, previously shown sensitive to doxorubicin (19). The cells were cultured in RPMI 1640 with 10% fetal bovine serum, supplemented with L-glutamine (200 mmol/L) and 2.2% of 100× penicillin-streptomycin, and incubated at 37°C and 5% CO_2. For tumor inoculations, the flanks of 6- to 8-week-old female BALB/c mice were shaved, prepped with alcohol, and injected s.c. with ~5×10^6 cells. The weight and condition of the mice were monitored daily, and tumor volumes were measured with a digital caliper (length × width × depth).

Pulsed-HIFU exposures. The custom pulsed-HIFU system used in the study (Focus Surgery) and the manner by which the tumors were treated were previously described (20, 21). The exposures had the following variables: spatial average, temporal average intensity (I_{SATA}) = 1,300 W/cm^2; 120 pulses; pulse repetition frequency = 1 Hz; duty cycle = 10% (100 ms ON and 900 ms OFF); raster spacing (in the X and Y dimension) of 2 mm. With this treatment regimen and the current preclinical test bed, a typical exposure for an entire tumor was 15 to 20 min. Prior work with these exposure variables showed temperature elevations in s.c. murine tumors on the order of 4°C to 5°C (19).

In vivo *doxorubicin delivery.* Tumors were grown bilaterally in the flanks of BALB/c mice and treated once they reached an approximate (±20%) size of 400 mm^3. In the first of two drug delivery experiments, mice were randomly assigned to one of four treatment groups: saline, free doxorubicin, NTSLs, or LTSLs (n = 5). Tail vein injections (volume = 100 μL) of saline or liposomes were first given at a doxorubicin dose of 2 mg/kg. At two time points following the injections (0 and 24 h), the mice received a pulsed-HIFU exposure in one of the two tumors; the non-exposed tumors served as internal controls.

Immediately after the exposures, the mice, still under isofluorane anesthesia, were euthanized by cervical dislocation and perfused by opening the chest cavity and giving a 10-mL i.c. injection of PBS to clear the vasculature of drug/liposomes. The tumors were excised immediately after perfusion and placed in pre-weighed 5-mL glass test tubes, which contained 2 mL of acidic ethanol (3% HCl, 48.5% ethanol, 48.5% DDI water). The tubes were weighed again to determine tumor weight, and the samples were ground up using a siliconized homogenizer. The homogenates were gently rotated overnight in the dark at 4°C. The next day, these were centrifuged at 5,000 rpm for 10 min at 4°C. Three 100-μL supernatant aliquots from each sample were then placed in an all-white 96-well plate and read with an LS-55 fluorimeter (Perkin-Elmer) for 1 s (excitation, 480; emission, 590). Fluorescence readings were compared with values from a standard curve comprised of serial dilutions of doxorubicin. The amount of doxorubicin in each tumor was normalized to its weight, and these were pooled and presented as group means.

In the second drug delivery experiment, the i.t. concentration of doxorubicin was determined with only the LTSLs, with or without pulsed-HIFU exposures. Mice were injected as in the first experiment; the pulsed-HIFU exposures, however, were given at 30, 60, or 120 min after injection (n = 5). Mice were sacrificed immediately after the exposures, and doxorubicin concentration was determined.

Effects on tumor growth. In this experiment, tumors were grown unilaterally in the right flank. When the tumors reached an approximate (±20%) size of 200 mm^3, the mice were randomly assigned to one of six experimental groups, where one of three injections (100 μL) were given (saline, NTSLs, or LTSLs), with and without pulsed-HIFU exposure (n = 6). In this study, the doxorubicin dose administered was 5 mg/kg, previously shown to be approximately half the dose required to produce significant growth inhibition in this tumor model with NTSLs containing doxorubicin (19). Pulsed-HIFU exposures were given immediately following the injections. Mice were monitored, and tumors were measured every second day posttreatment. The mice were sacrificed when tumors reached a size of at least 500 mm^3. The number of days to reach this size was pooled and presented as a group mean.

Statistics. For both drug delivery and growth studies, a Tukey-Kramer Honestly Significant Difference test was done (JMP software

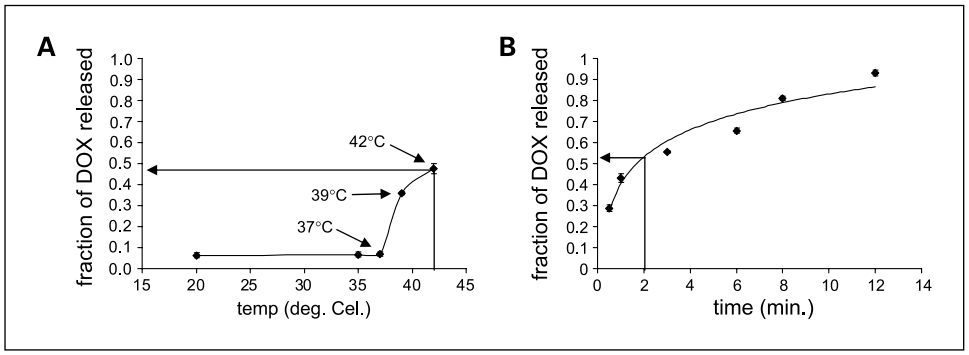

Fig. 1. Fraction of doxorubicin (*DOX*) release *in vitro* as a function of temperature (T) and time (t): (A) t = 2 min and T = 20°C to 42°C and (B) T = 42°C and t = 1 to 12 min. NTSLs did not release a detectable amount of doxorubicin even at the peak temperature of 42°C and the maximum incubation period of 12 min. For 2-min incubations, LTSLs started releasing doxorubicin at a temperature of 39°C, reaching almost 50% at 42°C. At a temperature of 42°C, release of doxorubicin at 2 min in LTSLs was ~50% and nearly 100% by 12 min. Points, mean (n = 4); bars, SE.

package) to determine if significant differences existed between each possible pair combination of experimental groups means. Significant differences between individual groups were determined by $P \leq 0.05$.

Results

Doxorubicin release from LTSLs is dependent on thermal dose. The goal of these experiments was to characterize the dynamics of doxorubicin release from the liposomes as a function of temperature and time. Even at temperatures of 42°C and the longest duration tested of 12 min, the NTSLs did not release any detectable levels of their encapsulated doxorubicin. For an incubation time of 2 min, the LTSLs began releasing doxorubicin at a temperature of 39°C (≈35% of payload) and released even more at 42°C (≈50%; Fig. 1A). Conversely, when the temperature was kept constant at 42°C, ~50% of the doxorubicin in the LTSL was released and approached 100% after 12 min. (Fig. 1B).

In vivo *doxorubicin delivery from LTSLs is triggered by pulsed-HIFU.* These experiments were designed to determine how much doxorubicin could be deployed locally by first administering the liposomes and then exposing the tumors to pulsed-HIFU. Exposures were carried at both 0 and 24 h after administration. At 0 h, combining the exposures with NTSLs showed no significant increase in mean doxorubicin concentrations when compared with NTSLs or LTSLs alone. However, when pulsed-HIFU was combined with LTSLs, a significant increase in doxorubicin concentration was found, which was 3- to 4-fold greater than the mean concentration of all other doxorubicin groups.

At 24 h, combining the pulsed-HIFU exposures with the NTSLs similarly showed no significant increase in mean doxorubicin concentrations when compared with NTSLs alone. However, these concentrations were now significantly higher than the LTSLs at 24 h, with and without exposures. Mean doxorubicin concentrations in the two LTSL groups (with and without exposures) at 24 h were not significantly different. Although mean doxorubicin concentration in the NTSL groups increased significantly from 0 to 24 h, they were still significantly lower than that of the HIFU and LTSL group at 0 h.

Pulsed-HIFU exposures did not significantly enhance i.t. doxorubicin concentrations for administrations of free doxorubicin at 0 or 24 h. Levels of all four groups were not significantly different from each other and from those for the NTSLs at 0 h (Fig. 2).

A trend of increasing doxorubicin concentration was observed in tumors receiving LTSLs without pulsed-HIFU exposures, when the lag time between injections and assaying the tumors increased from 5 to 120 min. When pulsed-HIFU exposures were given, however, doxorubicin concentration among groups receiving the exposures at varying lag times (after the injections) were not significantly different (Fig. 3).

Enhanced delivery of doxorubicin with LTSLs and pulsed-HIFU improves antitumor effect. The goal of this experiment was to compare the time it took the tumors to reach a volume of 500 mm^3 between the two different liposomes, with and without the pulsed-HIFU exposures. Growth time was not found to be significantly different among groups receiving saline, saline and pulsed-HIFU, NTSLs, NTSLs and pulsed-HIFU, and LTSLs, being 5.7, 6.7, 6.5, 7.8, and 7.2 days, respectively. In comparison, tumors from the LTSLs and pulsed-HIFU group grew significantly slower, requiring 11.8 days to reach 500 mm^3 (Fig. 4).

Discussion

Pulsed-HIFU exposures have been used to enhance the delivery of different agents (small molecules, DNA, and nanoparticles) to a variety of tissue types, with consequent

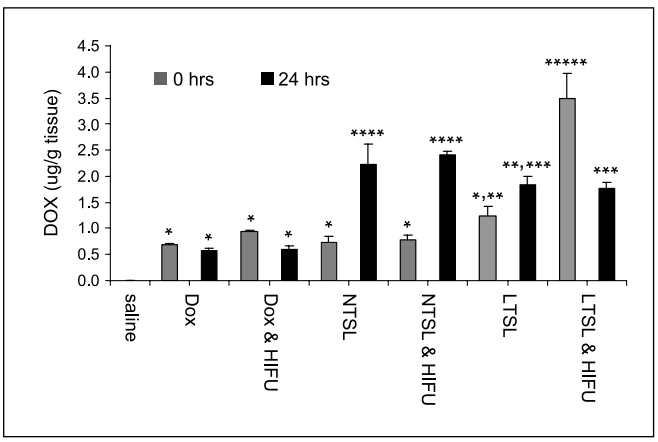

Fig. 2. Local drug delivery in murine adenocarcinoma tumors using free doxorubicin, NTSLs, or LTSLs, with or without pulsed-HIFU exposures. Liposomes or free doxorubicin (2 mg/kg) were first injected i.v. followed by exposures in the tumors (400 mm^3) at 0 and 24 h after administration. Immediately after the exposures, animals were sacrificed, and tumors were assayed for doxorubicin content. Significant differences were not found between exposed and unexposed tumors in mice receiving NTSLs at either exposure time point. The same occurred for free doxorubicin. Although accumulated doxorubicin was greatest in unexposed tumors receiving NTSLs (at 24 h), the highest mean concentration of doxorubicin was found in tumors receiving LTSLs and pulsed-HIFU exposures. Differing lowercase letters between mean doxorubicin concentrations indicate a significant difference of at least P = 0.05. Columns, mean (n = 5); bars, SE.

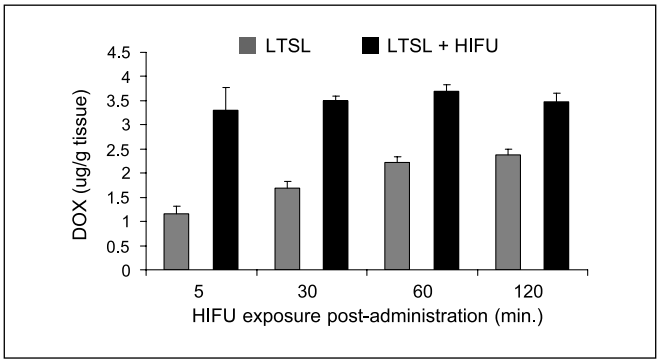

Fig. 3. Local drug delivery in murine adenocarcinoma tumors using only LTSLs, with or without pulsed-HIFU exposures. Lag time between i.v. liposome injection and exposures in tumors (400 mm^3) was varied. Immediately afterwards, animals were sacrificed, and tumors were assayed for doxorubicin content. Whereas doxorubicin in unexposed tumors continued to accumulate with time, lag time between injection and exposures in tumors did not affect content of doxorubicin. Columns, mean (n = 5); bars, SE.

increased therapeutic efficacy (20). Preliminary experimental and theoretical data indicate that these exposures produce primarily non-thermal, mechanical effects, such as local radiation force–induced displacements and consequent shear that can alter the permeability of the tissues and improve drug delivery. Typically, the exposures are given before administering an agent, where reversal of the enhancing effects can take up to 48 h, depending on the size of the injected material (22, 23).

In contrast to these results, a previous pulsed-HIFU study using NTSLs (Doxil) produced neither increased drug deposition nor improved growth inhibition in a murine adenocarcinoma breast cancer tumor model (19). Liposomes are designed to preferentially accumulate in tumors compared with non-tumor tissue (24), which was consistent with the findings of the study. The lack of observable effects on delivery enhancement normally produced by pulsed-HIFU exposures was consequently shown to be due to the liposomal formulation of the NTSLs (which inherently improves penetration into tissue) because an increase in delivery was induced and detected with similarly sized, non-liposomal nanoparticles and pulsed-HIFU. The study further showed the exposures do not produce damage to the treated tissues, nor a reduction in tumor growth, where minimal temperature elevations of only 4°C to 5°C occur (19).

The LTSL formulation tested here is undergoing a phase I multi-institutional study in combination with radiofrequency ablation for unresectable liver tumors.[3] Preclinical work in small and large animal models with radiofrequency ablation as the source of hyperthermia showed markedly increased drug deposition in tissue reaching the deployment temperature threshold, compared with unheated tissue, or compared with heated tissue in the presence of circulating free drug.[3] The present study attempted to determine if nonharmful temperature elevations produced by pulsed-HIFU exposures could be combined with LTSLs to increase doxorubicin delivery in targeted tumor tissue and, in that way, potentially improve the drug's efficacy. In previous drug delivery studies, pulsed-HIFU exposures were administered before therapeutic agents (19, 21). In contrast, to use the heat produced by pulsed-HIFU to release the doxorubicin payload from the LTSLs, liposomes were administered before exposures.

The pulsed-HIFU exposures were 2 min in duration at each individual raster point, with the transducer rastering from point to point to treat the entire targeted tissue. Temperature elevations reached peak levels within seconds after the exposure commences and returned to baseline (i.e., body temperature) exponentially within minutes after the exposure ends (19). Preliminary *in vitro* experiments were carried out to validate that the thermal dose (i.e., temperature elevation × duration) occurring during the pulsed-HIFU exposures could be used to trigger the release of doxorubicin from LTSLs. The results showed that doxorubicin did begin to be released from LTSLs at 39°C (similarly described by ref. 13), and that a substantial fraction (~50%) of the doxorubicin could be released when heated to 42°C for a period of 2 min. As expected (13), doxorubicin was not released in detectable levels from the NTSLs.

In subsequent *in vivo* experiments, combining the exposures with the LTSLs was found to significantly enhance local doxorubicin concentration in the targeted tumors, where no enhancement occurred with the NTSLs (i.e., no differences were found between treated and untreated tumors) or the LTSLs without the exposures. These results supported those found *in vitro*. When the short-term lag time between LTSL injection and pulsed-HIFU exposure was increased (from 5 to 120 min), the resulting enhancement remained constant, validating the dependency on the hyperthermia source for triggering release of the drug. The release of drug from LTSLs with pulsed-HIFU was indeed very rapid as tumors assayed for doxorubicin immediately following the exposures (0 h) showed significant enhancement of i.t. concentration of doxorubicin compared with non-exposed controls. The manner by which the triggering of drug release is dependent on hyperthermia has previously been reported (9), where the authors visualized the release of doxorubicin from TSLs in the presence of hyperthermia and also showed how this release ceases with the removal of the hyperthermia source.

Similar to previous published studies using liposomal doxorubicin without hyperthermia (19), the present study showed that over time, doxorubicin gradually increased in tumors when both NTSLs and LTSLs were used without pulsed-HIFU. However, even at 24 h, levels of doxorubicin with either liposomal formulation were still significantly lower than that of tumors receiving both pulsed-HIFU exposures and the LTSLs. That doxorubicin concentrations with NTSLs at 24 h were found to be significantly higher than with the LTSLs (without HIFU) was not surprising. In contrast to the LTSLs, the NTSLs possessed polyethylene glycolylated membranes, allowing for a smaller volume of distribution and increased circulation time, consequently leading to enhanced accumulation in tumors over time (6). When pulsed-HIFU exposures were given at 24 h with the LTSLs, doxorubicin concentrations were not significantly different than without the exposures. These results seem to indicate that the LTSLs were no longer present in the circulation at sufficient levels to be triggered by the hyperthermia source.

Significantly slower growth rates of tumors receiving LTSLs and pulsed-HIFU exposures (given at 0 h) could be explained by an overall increase in delivery of doxorubicin; however, faster delivery of the drug was also observed and may have also

[3] Unpublished data.

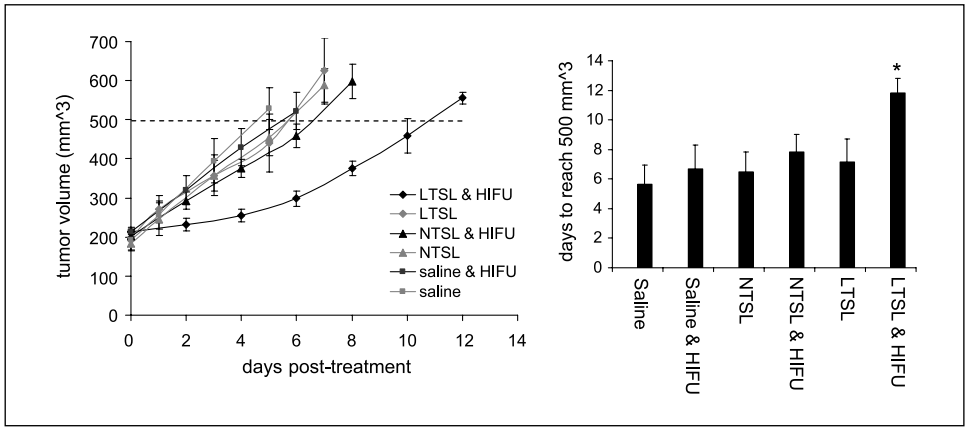

Fig. 4. Left, growth curves for mice receiving saline, NTSLs, or LTSLs, with or without pulsed-HIFU exposures. Tumors were treated (doxorubicin, 5 mg/kg) when they reached a size of 200 mm^3, and the mice were sacrificed when the tumors reached a size of at least 500 mm^3. Right, number of days posttreatment for tumors to reach 500 mm^3. Tumors receiving LTSLs and pulsed-HIFU exposures grew significantly slower than tumors in all other groups ($P < 0.05$). Points/columns, mean ($n = 6$); bars, SE.

contributed to the improved antitumor effect. Whereas dose dependency of the therapeutic effects of doxorubicin has been well documented (25, 26), Kong et al. (13) showed that triggered release of doxorubicin from TSLs results in greater concentration of i.t. doxorubicin, more pervasive distribution of doxorubicin in the tissue, and ultimately greater antitumor effect.

Past studies on pulsed-HIFU have shown the ability of similar exposures to enhance the delivery of various macromolecules (20), however, not the delivery of NTSLs (19). In these studies, the exposures preceded i.v. administration of various agents, which consequently experienced little or no influence of the minor and transient temperature elevations occurring during the exposures. Recent studies have indeed shown that using a non-HIFU heat source with the same thermal dose of the pulsed-HIFU exposures does not enhance the delivery of agents.[4] In the present study, exposures were given after administration of the liposomes (which continued to circulate in the blood); however, still no enhancement was seen in the delivery of doxorubicin with the NTSLs. Previous studies have shown that similar levels of hyperthermia (42°C) can increase the delivery of NTSLs by increasing tumor blood flow and vascular permeability and consequently improve the deposition of doxorubicin and its antitumor effect (13). In that study, however, hyperthermia was administered for 1 h compared with 2 min (per pulsed-HIFU raster point) in the present study. These relatively short exposures did not enhance delivery of conventional (NTSL) liposomes, which supports the assertion that the triggering of doxorubicin release from the LTSLs was due to locally induced hyperthermia and not previously described non-thermal mechanisms for improving drug delivery with pulsed-HIFU (20).

One of the major advantages of HIFU as a source of hyperthermia is that the exposures are noninvasive and applied extracorporally, compared with other types of hyperthermia like radiofrequency ablation or microwave ablation, which require interstitial needle or antenna insertion. Using image guidance, the focal zone can be positioned accurately at the targeted tissue (even deep with the body), where the beam passes over the skin and underlying tissues over a wide area and hence produces little or no heat (27). The drawback of this type of targeting, however, is that relatively small volumes of tissue are treated at one time in comparison with, for example, microwave hyperthermia. However, new and emerging technologies are being developed that could markedly increase the rate of HIFU-induced hyperthermia and therefore reduce treatment time. One example is the split beam–focused transducer, which has been shown to heat volumes of tissue 3- to 4-fold greater than a single focused beam within the same time period (28). Another is the use of focused phased-array transducers (29), whose focus can be positioned and redirected much faster than traditional mechanical means, potentially treating multiple, adjacent regions of tissue during the relatively long "OFF" part of the pulse cycle.

Image-guided HIFU for drug delivery (or tissue destruction) can be targeted to specific tumor tissue with ultrasound, computed tomography, or magnetic resonance imaging guidance and feedback. Each imaging modality may also provide thermometry of varying sensitivities. The tailored design of TSLs in combination with an image guided energy source bridges the gap between diagnostic imaging and therapeutic medicine. Combining therapeutic and diagnostic agents within the same liposome, for example, could provide surrogate markers of drug delivery, sensitivity, or efficacy long in advance of tumor response, facilitating patient-specific and tumor-specific drug selection. As a research tool, this could also facilitate drug discovery.

In conclusion, we have shown for the first time that pulsed-HIFU exposures can be combined with LTSLs to noninvasively produce enhanced and more rapid local delivery of doxorubicin in tumors, compared with NTSLs, resulting in improved antitumor effects of the drug. Future studies employing more advanced HIFU systems for both spatially and temporally improved hyperthermia (30), magnetic resonance contrast agents loaded into TSLs for *in vivo* monitoring of tissue pharmacokinetics (11, 12, 31) and hence more efficient optimization of hyperthermia, and newer generation of LTSLs that deploy drug more efficiently (32) may lead to further refinements in targeting and delivery of drugs. Energy-deployed drugs and image-guided therapies could be administered together and tailored to further enhance drug delivery, potentially widening otherwise narrow therapeutic windows and resulting in a new way to approach targeted rational design of patient-specific cancer therapies.

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[4] Frenkel, personal communication.

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