Cancer Chemother Pharmacol (2009) 64:593–600
DOI 10.1007/s00280-008-0910-8

ORIGINAL ARTICLE

Role of frequency and mechanical index in ultrasonic-enhanced chemotherapy in rats

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Received: 25 August 2008 / Accepted: 15 December 2008 / Published online: 7 January 2009

Abstract
Purpose The therapeutic effect of ultrasound and micellar-encapsulated doxorubicin was studied in vivo using a tumor-bearing rat model with emphasis on how tumor growth rate is affected by ultrasonic parameters such as frequency and intensity.
Methods This study employed ultrasound of two different frequencies (20, 476 kHz) and two pulse intensities, but identical mechanical indices and temporal average intensities. Ultrasound was applied weekly for 15 min to one of two bilateral leg tumors (DHD/K12/TRb colorectal epithelial cell line) in the rat model immediately after intravenous injection of micelle-encapsulated doxorubicin. This therapy was applied weekly for 6 weeks.
Results Results showed that tumors treated with drug and ultrasound displayed, on average, slower growth rates than non-insonated tumors ($P = 0.0047$). However, comparison between tumors that received 20 or 476-kHz ultrasound treatments showed no statistical difference ($P = 0.9275$) in tumor growth rate.
Conclusion Application of ultrasound in combination with drug therapy was effective in reducing tumor growth rate, irrespective of which frequency was employed.

Keywords Ultrasound · Drug delivery · BDIX rats · Tumor model · Micellar drug carrier · Mechanical index · Ultrasonic frequency

Abbreviations

Dox	Doxorubicin
IP	Intraperitoneal
MI	Mechanical index
SC	Subcutaneous
TV	Tumor volume
US	Ultrasound

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Introduction

The use of chemotherapeutic drugs is a common treatment for solid cancerous tumors. Unfortunately, these drugs are often not selective between healthy and cancerous cells. Therefore, it is difficult to optimize the balance between administering enough drug to destroy the cancer and giving so much drug that it causes patient morbidity and mortality. These drawbacks to conventional chemotherapy lead many investigations to seek novel ways to locally deliver the drugs selectively to the tumor, thus eliminating damaging exposure to healthy tissues.

There are several novel nano-sized carriers that have been used for ultrasonic-enhanced drug delivery [1]. These include micelles, liposomes and polymeric nanoparticles.

Micelles are usually used to deliver hydrophobic drugs because they can be easily sequestered in the hydrophobic interior of the micelle [2]. They are usually polymeric in nature, and have been combined with ultrasound (US) to treat solid tumors with anthracycline drugs [3–7]. Liposomes differ from micelles in that their drug payload is usually sequestered in their aqueous interior [8]. Liposomes containing doxorubicin have been combined with ultrasound to treat solid tumors [9–11]. Nanoparticles containing (or consisting of) drugs are usually designed to be small enough to extravasate beyond the endothelial boundary of capillaries [12, 13]. Ultrasound is used to enhance the extravasation process.

Ultrasound has also been used for gene delivery, usually in conjunction with liposomes and polymeric nanoparticles [14–16]. For example, the group of Hosseinkhani has shown effective ultrasonic-enhanced gene delivery using polyplexes of DNA and cationic-derivatized natural polymers, such as cationized gelatin [17–20] and dextran [21]. The authors speculated that cavitation-induced cell membrane damage and permeation were responsible for the enhanced genetic expression.

This paper presents our research in which chemotherapeutic drugs are loaded inside stable micelle carriers and injected into the circulatory system of a rat. Ultrasound is then used to locally deliver the toxic drug by permeabilizing the cell membranes of cancerous tissue while simultaneously disrupting the micellar structure to release the drug [1]. This treatment ideally has the potential both to preclude toxicity away from the target tumor and to locally increase the drug concentration in the cancerous tissue, thus increasing the drug's efficacy. However, specific challenges remain, and the delivery still needs to be optimized. Important issues to address include whether different frequencies and intensities of ultrasound affect the delivery of the drug and whether this delivery system is effective in treating cancer [22]. The purpose of this article is to address some of these issues and provide a greater understanding of ultrasonic drug delivery from micelles.

Our research uses stabilized polymeric micelles to carry doxorubicin (Dox) through the blood system of a rat, whose hind legs host colorectal epithelial solid tumors. Ultrasound is then applied to only one of the tumors. Ultrasound consists of pressure waves with frequencies greater than 20 kHz, generated by transducers that change a voltage waveform into mechanical movement of the transducer face. Like optical and audio waves, ultrasonic waves can be focused, reflected, refracted, and propagated through a medium [23]. Therefore, ultrasonic waves can be directed to and/or focused on a specific tissue area—a useful property that makes ultrasonic therapy minimally invasive (no surgery required).

Ultrasound intensity is the amount of power delivered by the pressure wave per unit area, commonly measured in watts per square centimeter (W/cm^2). As ultrasonic intensity increases, gas bubbles form and oscillate in the ultrasonic pressure field, a phenomenon called cavitation. At sufficiently high intensities, the bubble oscillations become unstable, and the inertia of the fluid can cause the gas bubble to collapse into a small volume of highly compressed and heated gas [24]. This type of cavitation, called inertial or collapse cavitation, can be stressful to cells because of the very high shear stresses in the region of the collapse, the concentrated energy of the shock wave produced by the bubble collapse, and the free radicals produced by the high temperatures. Furthermore, if the collapse is near a solid surface or even the semi-solid surface of a cell, an asymmetrical collapse occurs, which ejects a jet of liquid at sufficient speed to pierce nearby cells [25, 26].

In general, the likelihood and intensity of collapse cavitation increases at higher intensities and lower frequencies [25] and is indicated by the "mechanical index" (MI), the ratio of peak negative pressure, P^-, (in MPa) to the square root of frequency, f, (in MHz): [27]

$$\mathrm{MI} = \frac{P^-/\mathrm{MPa}}{\sqrt{f/\mathrm{MHz}}} \tag{1}$$

The threshold for collapse cavitation occurs at about $\mathrm{MI} = 0.3$, biological effects are observed at $\mathrm{MI} > 0.6$, and tissue damage is often observed when $\mathrm{MI} > 1$ [27, 28].

In the search for better cancer treatment methods, new innovations in micellar drug delivery have emerged with successful preliminary results. A micellar aggregate of polymeric surfactant creates a hydrophobic core and a hydrophilic corona. Micelles carry hydrophobic chemotherapeutic drugs within their cores and can deliver these drugs directly to target areas. In vitro release from our micellar carriers is induced by ultrasound when the $\mathrm{MI} \geq 0.38$ [4, 29].

Ultrasound has been shown to trigger the release of Dox from Pluronic® micelles at low frequencies [5, 7, 30–33]. Munshi et al. [34] were the first to report that ultrasound enhanced the uptake of Dox from micelles to a human leukemia cell line. In vitro studies by Husseini et al. [30] showed that 70 kHz ultrasound released about 10% of the drug from the micelles, and that after cessation of insonation, the drug was quickly re-sequestered in the micelle. Marin et al. studied the uptake and distribution of doxorubicin released from Pluronic® micelles. They concluded that there are two different mechanisms involved. First, ultrasound releases the drug from the micelles, causing a higher local concentration than observed without ultrasound. Second, ultrasound perturbed the cell membranes, which resulted in more accumulation of drug inside the cells [33].

Nelson et al. [35] employed an in vivo rat model to investigate the effects of ultrasonically controlled release of micelle-encapsulated doxorubicin. The particular micellar carrier used in that work consisted of polyether triblock surfactants stabilized by an interpenetrating network of a thermally sensitive acrylamide [36]. In that study, Dox in the carrier and ultrasound were applied weekly for 4 weeks. The tumors were exposed to 20 or 70-kHz ultrasound for 1 h following each weekly dose. Results showed that application of low-frequency ultrasound and encapsulated Dox at concentrations of 2.67 mg/kg resulted in a significant decrease in tumor size compared to controls [35].

The present study goes beyond Nelson's experiments to address some significant unresolved issues and to identify possible mechanisms. Specifically, our study held the mechanical index and time-averaged power intensity constant in order to solely investigate the effect of frequency on the therapeutic effect of Dox from stabilized micelles.

Materials and methods

In this experiment, 24 rats were treated for six consecutive weeks. Once a week, each rat received a systemic injection of micellar-encapsulated Dox followed by ultrasound treatment. All procedures involving rats followed NIH guidelines for humane animal use and care, and were approved by IACUC of Brigham Young University.

Tumor model

The BDIX rat readily grows the DHD/K12/TRb colorectal epithelial cancer cell line, which is susceptible to Dox [37]. This cell line can be injected nearly anywhere in the rat to successfully produce tumors [38]. Tumors were grown by intradermal injection of 25 µl of cells (2×10^6 cell/ml) in both rear legs of 24 female BDIX/CRCrl rats (Charles River Laboratories, Wilmington, MA) as described in Nelson et al. [35]. Tumors were apparent at the inoculation site within 2–3 weeks.

Drug/micelle preparation

The drug carrying micelles used herein were stabilized Pluronic® P105 micelles that were synthesized as described previously and stored at −20°C until use [36]. These micelles, called NanoDeliv™, consist of a hydrophobic core of polypropylene oxide and an outer corona of polyethylene oxide, stabilized using an interpenetrating network of thermally responsive acrylamide. At room temperature, the average diameter is about 125 nm. Doxorubicin was loaded into these micelles by introducing 3.75 ml of stabilized micelle suspension into a 10-mg vial of Dox via a 0.22-µm membrane filter (for sterilization). The Dox/micelle suspension was stored at −20°C and only thawed for short periods at the time of injection.

Encapsulated Dox injection

Prior to Dox injection, rats were tranquilized initially with ketamine (55 mg/kg, IP) and then pretreated with dexamethasone (4.0 mg/kg, SC) and diphenhydramine (5.0 mg/kg, SC) injections to reduce the risk of anaphylactic shock. The hind legs and tail were shaved, and the hair at the tumor site receiving ultrasound was completely removed through application of depilatory cream (Nair®) for 60 s. Lubricating ophthalmic ointment was administered to prevent the rats' eyes from drying during the anesthetic period.

Administration of the encapsulated Dox at 2.67 mg/kg (rat body mass) was given via a 25 g needle winged infusion set (Terumo, Somerset, NJ) in the lateral tail vein, followed by 3 ml of physiologic saline to completely flush the drug from the catheter. Rats were immediately placed in ventral recumbency and observed for adequacy of breathing.

Ultrasound application

Following the injection of Dox, the rats were sedated with the alpha-2 agonist medetomidine (0.3 mg/kg, IP), which when combined with the previously administered ketamine, produced adequate sedation and muscle relaxation for the procedure. Medetomidine administration was necessary to prevent the rats from moving their legs while ultrasound was applied to the tumor.

Approximately 5 min after infusion, ultrasound treatment was applied for 15 min to one tumor using either a 20-kHz probe (Vibra-Cell; Sonics & Materials, Inc., Newtown, CT) or a 476-kHz transducer (Sonic Concepts, Woodinville, WA). The second tumor (on the contralateral leg) was not exposed to ultrasound and thus served as the internal control. Half of the rats were insonated using the 20-kHz probe (continuous wave, intensity of 1.0 W/cm^2, pressure amplitude of 0.173 MPa). The mechanical index at the tip of the 20-kHz transducer operating at 1.0 W/cm^2 was 1.22. The other 12 rats were treated using the 476-kHz transducer. In order to match the mechanical index (1.22) while using 476-kHz US, an intensity of 23.61 W/cm^2 (pressure amplitude of 0.842 MPa) was required. However, transmitting this amount of energy would most likely cause thermal damage to the rat tissue. Therefore, the 476-kHz transducer was pulsed (1,000 cycle) at a pulse repetition frequency of 20.161 Hz (duty cycle of 0.0424) to create a temporal average intensity of 1.0 W/cm^2. Thus both

ultrasonic applications had the same mechanical index of 1.22 and the same temporal average power density of 1.0 W/cm^2.

For insonation at 20 kHz, ultrasound-conducting gel (Aquasonic 100, Parker Laboratories, Fairfield, NJ) was applied on the skin of the leg above the tumor, and the 20-kHz probe was placed in this gel but not directly touching the skin. For insonation at 476 kHz, ultrasound-conducting gel was applied on an acoustically transparent window of polypropylene film (38 μm thick, Exxon Chemical, Mar-Lin, PA) at the focal point of the 476-kHz transducer. The targeted tumor was placed in the gel at the focal point, and the leg was immobilized with tape applied at the tarsus below the tumor site.

Tumor growth measurement

After 15 min of insonation, the tumor sizes were measured, and the rats were injected subcutaneously with atipamizole (84.0 μl/kg), to reverse the effect of medetomidine. Each tumor was measured by making two perpendicular measurements (a and b, with $a \geq b$) using calipers. Tumor volume (TV) was then determined using the formula [38]:

$$\mathrm{TV} = \frac{a \cdot b^2}{2} \tag{2}$$

In some cases, it became difficult to accurately measure the size of the tumor. Skin covering the area of interest made it difficult to determine the exact edges of the solid mass. Therefore, each measurement was taken three times to help decrease this "noise" and increase the reliability of the statistical analysis.

Statistical procedure

The tumor volume data (from insonated and control tumors) were analyzed using an exponential growth model:

$$\mathrm{TV} = A_0 \mathrm{e}^{kt} \tag{3}$$

where t is the time after the first drug injection, TV is the tumor volume at time t, A_0 is the tumor volume immediately following the first treatment ($t = 0$), and k is the tumor growth rate constant.

Tumor volumes were transformed to the natural logarithmic scale:

$$\ln(\mathrm{TV}) = \ln(A_0) + kt \tag{4}$$

Using SAS software (SAS Institute Inc., Cary, NC), log-transformed volumes were then analyzed using a linear mixed model with ultrasound treatment, frequency, days after initiation of treatment, and all possible interaction of these factors as fixed effects. Initial tumor volumes, rat-specific tumor growth rates, and repeated volume determinations were considered to be random effects. The model was fitted using residual maximum likelihood for the variance components and estimated generalized least squares for the fixed effects. Residuals were computed and plotted versus predicted values to assess goodness-of-fit of the model. Main effects and interactions of the fixed effects were tested using approximate F tests based on the Kenward–Roger adjustment for small-sample inferences.

Results and discussion

Our in vivo rat model was employed to investigate a novel tumor treatment involving the localized delivery of a chemotherapeutic drug (Dox) using stabilized Pluronic® micelles as the drug carrier and low-frequency ultrasound as an external triggering mechanism to release the drug directly at the treatment site. As part of this research, the effects upon tumor growth were successfully studied using different ultrasonic frequencies but identical values for intensity and MI.

Of the 24 rats initially used in the experiment, 23 survived all 6 weeks of the study. One rat from the 476-kHz group died during the first day of treatment. Autopsy results for the rat that died implied hypothermia to be the cause of death, not the treatment or the cancer. The hypothermia was thought to have occurred because the rat was not completely dried after being washed to remove the depilatory lotion. As the rat was under anesthesia, its ability to control body temperature was compromised. There were no other complications during the experiment. Most of the rats, however, became lethargic and started to lose substantial weight (greater than 10% over a week) after the fifth week of treatment. It is suspected that this was due to the combination of the chemotherapy and the growing tumors, some of which had metastasized to other locations. Necropsy on a subset of the rats showed that all animals sampled had metastatic lesions in the lungs and abdominal lymphatic system (9 of 9), irrespective of whether they received 20 or 476-kHz US.

The tumor growth (or recession) rate was adequately modeled by an exponential growth rate, for both control and insonated tumors at both frequencies. Comparison between tumors that received 20-kHz US and those that received 476-kHz ultrasound showed no statistical difference ($P = 0.9275$) in tumor growth rate. We could not reject the null hypothesis that the tumors grew at the same rate with 20 and 476-kHz insonation.

Because there was no difference attributed to the parameter of frequency, the data from both frequencies were combined to examine the differences in growth rate for the insonated tumors versus control tumors. The exponential growth rate constant for the non-insonated

tumors ($k_{control}$) was 0.0465/day (standard error = 0.0066), while that for both frequencies of insonated tumors ($k_{ultrasound}$) was 0.0402/day (standard error = 0.0066). The difference was 0.0063/day (standard error = 0.0022). Comparison between the growth rate constants of the insonated tumors and non-insonated tumors showed that the insonated tumors displayed significantly slower growth rate ($P = 0.0047$). The percent growth inhibition, defined as $\{1 - k_{insonated}/k_{control}\} \times 100$, was 13.5%. Figure 1 shows an example of tumor growth data collected from one of the rats in the study (from the 476-kHz group). These particular rat data were chosen because they are representative of the statistical average results of the study. Within the group that received 20-kHz US, the treated growth rate constant was 0.048 ± 0.007/day for treated tumors and 0.053 ± 0.005/day for untreated tumors. Within the group that received 476-kHz US, the treated growth rate constant was 0.032 ± 0.013/day for treated tumors and 0.038 ± 0.007/day for untreated tumors.

In addition to the overall group comparisons described above, a paired comparison statistical analysis was done in which the growth rate of the treated tumor was compared directly to the growth rate of the contralateral untreated tumor in the same rat. This analysis showed that for rats in the 20-kHz group, the hypothesis that the insonated tumors grew at the same rate was very close to the boundary of being rejected ($P = 0.057$). However, in the 476-kHz group, we could reject the null hypothesis and state that the treated tumor grew slower than the contralateral non-insonated control in the same rat ($P = 0.028$). When combining both groups, the treated tumor definitely grew slower than the contralateral control in the same rat ($P = 0.006$). This is consistent with other similar studies in rats and mice [3, 35, 39].

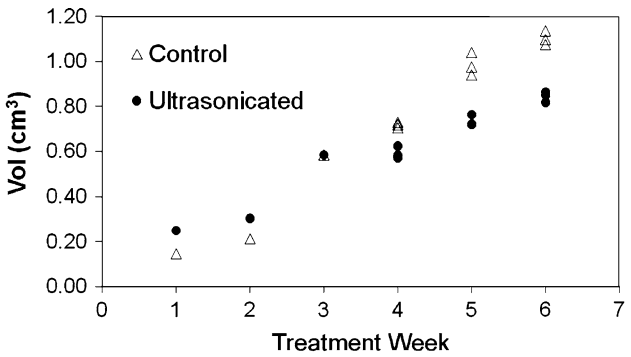

Fig. 1 Tumor volume data collected from one of the rats (from the 476-kHz group) in the study. This graph is representative of the overall results of the study and has growth rate similar to the average of the 476-kHz group: a small, but significant decrease in the growth rate of tumors exposed in vivo to low-frequency ultrasound, after its host was infused with micellar-encapsulated doxorubicin. Key points to notice are the different initial volumes at week one and the variations in measured volumes and growth trend throughout the study. The *triangles* represent the measured volumes of the tumor that did not receive ultrasound. The *circles* represent the measured volumes of the tumor that received ultrasound. Larger tumors were measured in triplicate to increase the reliability of the statistical results

For example, in a similar study Nelson et al. [35] used the same in vivo rat model to investigate the effects of ultrasonically controlled release of micelle-encapsulated Dox. During the course of their 4-week treatment, the volumes were measured for tumors exposed to 20 or 70-kHz ultrasound for 1 h. The comparison of the final tumor volumes showed that the insonated tumors had grown less at the 4-week endpoint than tumors that were not exposed to ultrasound. However, not enough data were available to show any statistically significant difference that could be attributed to the effect of 20 versus 70-kHz ultrasound [40]. The current project, however, is more thorough because not only did it increase the sample size, but also compared all measured tumor sizes throughout the 6 weeks of treatment and determined exponential growth rate constants for both insonated and non-insonated tumors.

We employed 15 min of insonation in the present study, which is less than the 60-min insonation exposure of our previous study [35]. Preliminary studies showed that 15 min of insonation were as effective as 60 min [41]. We are planning to investigate the effect of various insonation times in future studies.

One must be careful in interpreting the result that there is no statistical difference in tumor growth rates attributed to the parameter of frequency at 20 and 476 kHz. Such a result does not prove that a difference does not exist. It only means that in this experimental set there is no sufficient evidence to reject the null hypothesis that the growth rates at the two frequencies are the same. It is still possible that the growth rates are influenced by the frequency, but if so, any difference could not be revealed through the noise and scatter in this data set of 23 rats.

Although it is not surprising that ultrasound combined with Dox delivery from a polymeric micelle suppressed the tumor growth rate, it was noteworthy that the frequency of ultrasound had no statistically significant bearing on the result. In general, ultrasound at 20 kHz (low frequency) is used for very different applications than ultrasound at 476 kHz (mid frequency). Low-frequency ultrasound is absorbed poorly by tissues, is more difficult to focus (because of the much larger wavelength), and is used primarily for cell disruption and for the cleaning of small objects [42]. Mid-frequency ultrasound is absorbed much more readily by tissues and is therefore used in physical therapy applications to heat tissues [43], and in therapeutic applications to ablate and destroy tissue, such as high intensity focused ultrasound (HIFU) [44]. In potential clinical applications of this technology, the frequency of application is of great importance because low-frequency

ultrasound cannot be focused easily. Previous studies of transdermal drug delivery [45, 46] and antibiotic delivery to biofilms [47, 48] suggested that low-frequency ultrasound is much more favorable at a given power density because bubble cavitation was more pronounced at lower frequencies. The present results allow extension of this technology to mid-frequency ultrasound where focusing technology is available.

These experiments were specifically designed to investigate the effect of frequency by holding other important ultrasonic parameters constant. As described previously, the temporal average intensity was held constant at 1.0 W/cm^2 in both experimental sets, and the MI was held constant at 1.22. The MI is a measure of the likelihood and intensity of collapse cavitation. A higher value of MI is achieved at higher intensities and/or lower frequencies. Thus to match the MI values, a lower intensity was employed at 20 kHz without any pulsing, and a much higher intensity was applied in short pulses at 476 kHz. In both cases, the MI was 1.22, which is above the threshold for biological damage [27]. The observation that frequency makes no difference in tumor growth suppression supports the idea that the MI (which was held constant) may be a key factor in determining how much drug is delivered to the cells. Using a similar argument, it is possible that time-average intensity could also be a determining factor in tumor growth rate since that parameter was also held constant at 1.0 W/cm^2 in these experiments.

The observation that ultrasonic frequency (over the range studied) produced no measurable difference on tumor growth rate leads to important clinical implications. For example it is possible that future studies and clinical applications can use the most convenient ultrasonic frequency for drug delivery therapy to the tumors as long as the MI and power density are appropriate to generate positive results and yet to avoid collateral damage of healthy tissues. The effects of varying the MI and power density on tumor growth are still unknown but are the subjects of ongoing studies. A larger MI or power density could produce a stronger therapeutic effect, but that postulate remains to be tested.

This study corroborated previous studies by ourselves and others [3, 35, 39] that there is a beneficial and synergistic effect of combining ultrasound with chemotherapy. There may be many possible reasons for this favorable synergy. Although blood with the same concentration of drug perfused both bilateral tumors, the ultrasound may have released more drug from the micelles, depositing more Dox in the insonated tumor tissue. Although such a hypothesis is consistent with many in vitro studies [5, 31–34], release from micelles has never been demonstrated in vivo. As part of a concurrent pharmacokinetics study, the tumors from this study were removed and homogenized, and the doxorubicin was extracted and quantified [49, 50]. These other studies revealed an increased concentration of drug in insonated tumors within the first 30 min after treatment ($P = 0.055$). Thus we hypothesize that ultrasound creates a condition leading to a greater concentration of drug in the tumor [51].

Often the capillaries of tumors have enhanced permeability toward sub-micron-sized particles, similar in size to that of our micellar drug carrier [52, 53]. Thus it is possible that drug carriers might be accumulating in the tumor due to extravasation through leaky capillaries, often called the enhanced permeation and retention (EPR) effect [54]. Ultrasound may further enhance that permeability [55, 56]. If extravasation does occur, then it would be advantageous to allow the drug-laden micelles to accumulate in the tumor for several minutes to hours before application of the ultrasound to the tumor. Future studies should vary the time after carrier injection when the ultrasound is applied in an effort to leverage the EPR effect to further enhance tumor treatment.

A third possible biological mechanism at play in our results is that ultrasound may be enhancing the permeability of the cell membrane toward drug uptake, or even perhaps toward carrier uptake. Schlicher et al. [57] demonstrated that ultrasound facilitates uptake and retention of molecules present during insonation or introduced shortly after insonation ends. Also, they showed that cells exposed to ultrasound display membrane wounds or pores that were eventually repaired [26, 57, 58]. We believe that all three biological mechanisms proposed above contribute to US-enhanced tumor growth suppression.

It is also possible that, along with the lethal effect of Dox, the ultrasound lysed the cells or killed them indirectly via necrosis or stress-induced apoptosis. Low-frequency ultrasound alone at high enough intensities has been demonstrated to trigger cell-mediated death [59], but those intensities reported for cell death are much higher than those used in the experiments herein. Therefore we are not supportive for this mechanism until more supporting evidence is revealed.

While the statistical results showed that ultrasound improves the effectiveness of Dox delivery using micelles, the combined treatment failed to completely treat and cause regression of the cancerous tumors. Only 2 of the 23 tumors that received ultrasound completely regressed, and at least 1 of them is thought to have regressed mainly because of Dox therapy and the rat's immune system because the contralateral control tumor in this rat did not grow as fast as the control tumors in other rats. The other 21 insonated tumors continued growing in size, albeit at a slower rate than their contralateral control, on average.

Though this study showed that frequency has no effect on the treatment, other factors such as exposure time, MI,

number of treatments per week, and drug concentration in the micelles could be manipulated to improve the effectiveness. As mentioned, mechanical index is thought to be especially important in permeating cell membranes and releasing drugs from the micelles.

Conclusions

The combination of doxorubicin in micelles followed by the exposure to low-frequency ultrasound (at an intensity large enough to generate a high mechanical index) was effective in decreasing the tumor growth rate compared with non-insonated tumors. The tumor volumes were satisfactorily fitted to an exponential growth model where the growth rate constant for insonated tumors was 0.0402/day, while the rate constant for non-insonated tumors was 0.0465/day. However, different ultrasound frequencies (at the same mechanical index and time-averaged power density) showed no effect on tumor growth rate. These results implicate a key role for ultrasonic cavitation events, most probably through the release of drug from the micellar carrier, and through increased permeability of the cells and capillary walls.

Acknowledgment Funding for this research was provided by the NIH (CA98138).

Conflict of interest statement None.

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