Chemodosimetry of In Vivo Tumor Liposomal Drug Concentration Using MRI

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Effective cancer chemotherapy depends on the delivery of therapeutic drugs to cancer cells at cytotoxic concentrations. However, physiologic barriers, such as variable vessel permeability, high interstitial fluid pressure, and heterogeneous perfusion, make it difficult to achieve that goal. Efforts to improve drug delivery have been limited by the lack of noninvasive tools to evaluate intratumoral drug concentration and distribution. Here we demonstrate that tumor drug concentration can be measured in vivo using T1-weighted MRI, following systemic administration of liposomes containing both drug (doxorubicin (DOX)) and contrast agent (manganese (Mn)). Mn and DOX concentrations were calculated using T1 relaxation times and Mn:DOX loading ratios, as previously described. Two independent validations by high-performance liquid chromatography (HPLC) and histologic fluorescence in a rat fibrosarcoma (FSA) model indicate a concordant linear relationship between DOX concentrations determined using T1 and those measured invasively. This method of imaging exhibits potential for real-time evaluation of chemotherapeutic protocols and prediction of tumor response on an individual patient basis. Magn Reson Med 56: 1011–1018, 2006. © 2006 Wiley-Liss, Inc.

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Drug delivery systems, such as liposomes and polymers, have been used to improve the pharmacokinetics and biodistribution of chemotherapeutic agents after systemic administration. Liposomes are nanoscale drug carriers that consist of a spherical lipid bilayer with a diameter of <200 nm. One such carrier that has been approved for human use in cancer therapy is the Doxil™ liposome. This formulation is sterically stabilized and contains polyethylene glycol, which extends plasma half-life and reduces uptake by the reticuloendothelial system. A limitation to this formulation is that the sterical stability also causes the liposome to release the drug very slowly, which may not be ideal depending on the contents. We have examined a novel temperature-sensitive liposome (TSL) that undergoes membrane destabilization at 39.5–41.3°C, resulting in rapid site-specific drug release when combined with local hyperthermia (HT) (1,2). Our laboratory used the TSL formulation with local HT to dramatically increase tumor doxorubicin (DOX) concentration (up to 30-fold over free drug, and fivefold over what is achievable with a formulation similar to Doxil™). The resulting difference in drug concentration markedly improved the antitumor efficacy in preclinical models (3). The rapid drug release kinetics of this formulation may lead to nonuniform drug deposition because temperature distributions during HT are heterogeneous in tumors. However, with state-of-the-art heating technologies, temperature distributions can be altered during treatment. The ability to monitor drug delivery and release, coupled with spatial control over temperature distribution, would enable physicians to make more informed decisions during treatment and achieve the desired drug concentration distribution.

Recently we demonstrated that monitoring of liposomal drug delivery can be achieved with MRI and imageable liposomes. We developed nonthermal sensitive (NTSL, similar to Doxil™) and TSL formulations that encapsulated both DOX and manganese sulfate (MnSO4) (4,5). DOX was actively loaded into liposomes with the use of an MnSO4 buffer to create an ion gradient across the lipid bilayer and precipitate DOX on the interior (5). The resulting liposomes contained Mn2+ and DOX in a defined ratio (Mn2+/DOX = 0.42 ± 0.04 (ng/mg) / (ng/mg)). Since Mn2+ is a paramagnetic transition metal similar to gadolinium, it can be used as an MR contrast agent (6–8). In the same study (4) we described the in vitro properties of Mn2+-containing NTSL and TSL. Liposome relaxivity (i.e., the ability to act as a contrast agent; see Eq. [2]) was quantified for both intact liposomes and those whose contents had been thermally released. This enabled the development of an image analysis algorithm to calculate [DOX] from T1-weighted MR images (Fig. 1) (4).
To validate the MRI method, we administered liposomes (TSL and NTSL) to Fischer rats bearing fibrosarcomas (FSAs) grown in the flank, with and without 1 hr of local HT, using an MR-compatible heating device during imaging (9). Once thermal steady state was reached (15 min), liposomes were administered intravenously. A spoiled gradient-recalled echo sequence was chosen to minimize T1 effects while permitting extremely short acquisition times for 3D axial imaging of the whole-tumor volume. Differences in pre- and postcontrast T1 values were then converted to DOX concentration measurements.

T1-based [DOX] was validated by invasive methods in two independent studies. The first study used high-performance liquid chromatography (HPLC) with fluorometry to measure [DOX] after administration of TSLs (at various dosages) with local HT. The second study used tissue fluorescence intensity to measure [DOX] in frozen tumor sections treated with TSLs or NTSLs (at identical dosages) administered with or without local HT. This yielded information over a broad concentration range for both liposomal formulations.

MATERIALS AND METHODS

In Vitro Methods

Liposome Materials

Doxorubicin hydrochloride (DOX; Faulding, Quebec, Canada) was purchased from the British Columbia Cancer Agency, and 1,2 distearoyl-sn-glycero-3-phosphocholine (DSPC), 1,2 dipalmitoyl-sn-glycerol-3-phosphocholine (DPPC), 1-stearoyl-2-hydroxy-sn-glycerol-3-phosphocholine (MSPC), and 1,2 distearoyl-sn-glycerol-3-phosphoethanolamine-N(10) were obtained from Avanti Polar Lipids Inc. (Alabaster, AL, USA). Cholesterol (Chol), Sephadex G-50, and all other chemicals were obtained from Sigma Chemical Co. (St. Louis, MO, USA).

Liposome Synthesis and DOX Encapsulation

The liposome formulation and subsequent DOX encapsulation were previously described in detail (4,5). Briefly, the liposomes were prepared by extrusion methods and exhib-
ited an average diameter of 100–120 nm. NTSL was made from DSPC/Chol in a 55:45 molar ratio, and TSL was made from DPPC-MSPC-DPPE-PEG2000 (90:10:4). The liposomes contained 300 mM MnSO$_4$ at pH 3.5 to act as the contrast agent and to provide the salt gradient necessary for DOX loading.

The MnSO$_4$-based procedure for DOX loading was a modification of the method described by Abraham et al. (5). After the salt gradient was formed (by removing the MnSO$_4$ exterior to the liposomes by an exchange column), DOX was added to achieve a drug-to-lipid ratio (wt:wt) of 0.05:1, and loading was conducted at 37°C for 80 min.

In Vivo Methods

All procedures were approved by the Duke University Animal Care and Use Committee.

Tumor Transplantation

A methylcholanthrene-induced rat FSA tumor line was used for this study (4,9). The tumor line was maintained by serial transplantation. Tumors were allowed to grow subcutaneously in the flank to 17–20 mm diameter before use.

Animal Therapy

The rats were anesthetized with an IP injection of sodium methohexitol (Brevital) 45 mg/kg. Atropine sulfate was given IP (0.35 mg/kg) to prevent mucous secretion in the airways. An endotracheal tube (16–18 gauge intracatheter) was inserted and the rats were mechanically ventilated with isoflurane (1–3%). ECG electrodes were placed on the footpads and a rectal thermistor was used to monitor body temperature and provide feedback control of MRI bore air temperature (4). A 16-G catheter was placed through the center of the tumor. Heated water (50°C ± 0.1°C, 1.8 mL/s) was passed through the catheter to heat the surrounding tissue. The surface temperature of the catheter was 6°C cooler than the temperature of the heated water. The temperature profile has been determined to provide local HT to the entire tumor (T$_h$ = 39–44°C) (4,10,11). Tumors were heated for 15–20 min to reach thermal steady state before liposome injection (4). The rats were administered an intravenous dose of 7.5–12.5 mg TSL liposomal DOX/kg with heat when validated by HPLC, and 10 mg/kg of NTSL and/or TSL with or without heat when validated by fluoroscopy. This allowed us to extend the range of the study by showing the effect of HT, dosage, and liposome type.

MR Protocol

All MRI experiments were performed at the Center for In Vivo Microscopy at Duke University in a 2-T 30-cm bore diameter magnet (Sienna, GE Medical Systems, Milwaukee, WI, USA). During the MRI scans water was used for tumor heating and circulated through the indwelling catheter. The water was doped with 0.25 M MnSO$_4$ to prevent flow image artifacts through T$_1$ shortening (8). A spoiled gradient-recalled echo acquisition was used with a TR of 23 ms, TE of 1.4 ms, and flip angles of 2°, 5°, 8°, 10°, 12°, 33°, 46°, and 60° for the initial T$_1$ map. Subsequent drug uptake scans were performed at a 33° flip angle. The moderate flip angle, short TE, and short TR were selected to produce the greatest T$_1$ contrast. 3D volume images with 1–1.3-mm slice thickness were taken with a 6-cm field of view (FOV), yielding an in-plane pixel size of 234 × 234 μm. Number of excitations (NEX) = 1 (single image averaging) was used for the first 20 min, and NEX = 4 (four image averaging) was used for the remainder of the experiment for an improved signal-to-noise ratio (SNR). At the termination of the uptake scan, the local HT was discontinued. A second set of multi-flip-angle images were taken and a postcontrast T$_1$ image calculated.

Tissue Concentration Measurements

Ninety minutes after MRI the animals were killed and the tumors were excised. The heating catheter and a glass micropipette marker were kept in place. The tumor was snap-frozen with liquid N$_2$. Tissue sample DOX concentrations were measured by either HPLC or direct DOX histological fluorescence measurements. Nine animals were used for the HPLC study (four to eight tissue samples per animal, N = 48 samples), and 10 animals with one large region of interest (ROI) per animal (two for HT treated, an enhancing and nonenhancing region) were used for the tissue fluorescence study (N = 17 sampled regions).

HPLC for DOX Concentration

The frozen tumors (–80°C) were partially thawed and sliced in 3-mm slabs parallel to the MRI plane. A 4-mm biopsy punch was used to sample ROIs (50–100 mg per ROI). The heating catheter and glass micropipette facilitated image ROI registration; however, it was impossible achieve identical ROIs due to distortions in tumor structure during sampling. Each tissue sample was diluted and homogenized. Half of the homogenate was dried for Mn analysis. DOX was extracted from the remaining homogenate using chloroform and silver nitrate. The organic phase was separated, dried, and reconstituted in isopropanol. The DOX concentration was measured by HPLC with fluorometry. A standard concentration set was prepared using tumor homogenate and known concentrations of DOX. The overall sensitivity of this method was ±1 ng DOX per mg tissue.

Inductively Coupled Plasma Mass Spectrometry (ICP-MS) for Mn Concentration

Dried tissue samples were decomposed using 65% nitric acid and heated to 180°C for 4 hr. After dilution with high-purity water, the Mn content of each sample was measured by ICP-MS. The untreated tumor homogenate was analyzed for baseline Mn concentration. A set of bovine liver standards was prepared with known concentrations of Mn. The instrumental error for this method was ±3% of the total Mn content.

Fluorescence Image Analysis for DOX Concentration

The excised tumors were cut into four sections orthogonal to the heating catheter, mounted in OCT (Sakura Fineket,
Torrance, CA, USA), and snap-frozen with liquid N2. The center two sections were used for histology. Four consecutive 15-μm-thick sections were cut, fixed in 4% paraformaldehyde (PFA; Fisher Scientific International Inc., Hampton, NH, USA), and stored in 1% PFA. DOX fluorescence measurements were performed and slides were subsequently stained with hematoxylin and eosin. Fluorescence microscopy was performed using a Zeiss Axioscope microscope (Thornwood, NY, USA) with an automated shutter (Uniblitz, Rochester, NY, USA), a CCD camera with Andor image-capture software (South Windsor, CT, USA), and a Zeiss automated stage. The entire system was integrated with Labview (Austin, TX, USA) to facilitate time-controlled quantitative exposure of each field.

The slides were scanned with 0.03-s exposure time and a 5× objective lens over a range of 20 mm to cover the entire tumor slice. Fluorescence intensity was corrected for autofluorescence signal intensity and variation in excitation lamp intensity by using quantum dot slides (Evident Technologies, Troy, NY, USA) with a 580-nm emission. Quantum dot slides were imaged before, during, and after imaging the histology sections. Variations in lamp intensity during the imaging session were assumed to be linear, and DOX fluorescence was not adjusted since variation between imaging sessions was less than 5%. The corrected signal intensities were converted to DOX concentration based on a calibration curve derived from DOX dissolved in saline. DOX concentration was then measured for ROIs with geometry and location similar to those used in the MRI image analysis. However, it was impossible to achieve identical ROIs due to different image resolutions and distortions in tumor structure during processing.

MR Image Analysis

**T**\(_1\)-Based Concentration Measurements

We analyzed the MR images pixel by pixel using Matlab™, (Freeware code to perform most of this image analysis using image I plug-ins is available at http://dblab.duke.edu/.) We obtained the initial and final **T**\(_1\) and **S**\(_0\) maps by fitting the signal intensity at variable flip angles:

\[
signal = S_0 \sin(\alpha)(1 - \exp(- T_1/T_i)) \left(1 - \cos(\alpha)\exp(- T_2/T_i) \right)
\]

where **S**\(_0\) accounts for the proton density and **T**\(_2\) effect (8). Subsequent signal enhancement after injection of the liposomes was assumed to be caused by **T**\(_1\) shortening (8). Dynamic images obtained after contrast injection were converted to dynamic **T**\(_1\) maps based on the initial fit for **S**\(_0\) and using Eq. [1].

Changes in **T**\(_1\) from the initial **T**\(_1\) map were converted to concentration based on independently measured temperature distributions in the tumors and the temperature-dependent relaxivity of the Mn\(^{2+}\)-loaded liposomes (4). There is a linear relationship between the reciprocal of **T**\(_1\) and the MnSO\(_4\) concentration (8,12):

\[
\frac{1}{T_1^{\text{dynamic}}} - \frac{1}{T_1^{0}} = r_1(\frac{C_{\text{Mn}}^{\text{dynamic}}}{C_{\text{Mn}}^{0}})
\]

The slope (\(r_1\)) of this line is the “relaxivity” (mM*s)\(^{-1}\). However, since **T**\(_1\) is also temperature-dependent (8), the tumor must return to the body temperature of the animal before a postcontrast **T**\(_1\) map can be obtained for the most accurate measurement of concentration.

The TSL releases contents upon heating to temperatures ≥39.5°C, changing the interaction of Mn\(^{2+}\) with water. This increases the relaxivity. Therefore, knowledge of the temperature profile in the tumor is necessary to calculate Mn\(^{2+}\) concentrations during HT treatment. We previously published this temperature profile and the relaxivity results needed for these calculations (4).

Because of the complexity of liposome **T**\(_1\) dependence on temperature and/or Mn\(^{2+}\) encapsulation concentrations, we calculated all of the validation results shown with Eqs. [1] and [2] using the initial multiflip **T**\(_1\) measurement and the cooled (body temperature) tumor postcontrast multiflip **T**\(_1\) measurement. (Note: the use of the dynamic **T**\(_1\) measurement instead of the postcontrast multiflip **T**\(_1\) measurement yielded an underestimate of the Mn\(^{2+}\) concentration by 10–15%). The Mn\(^{2+}\) concentrations were then converted to DOX concentrations (μg/ml) based on the loading ratio of DOX to Mn\(^{2+}\) in the liposome (4). The molecular weight and encapsulation volume of the liposomes were also included in the calculations converting [Mn\(^{2+}\)] to [DOX] (4,5,13). ROIs for HPLC were selected in areas of high and low intensities to incorporate a DOX concentration ranging from 0 to 50 ng/mg.

Statistical Analysis

The relationships between invasive [DOX] and **T**\(_1\)-based [DOX] were analyzed at the sample or subject level data using the linear regression or mixed-effects model accounting for within-subject correlation when necessary (SAS®, SAS Institute Inc., Cary, NC, USA). The subject was a single animal. The sample consisted of a single ROI, which was sometimes averaged over a number of slices. The measurement was HPLC-, fluorescence-, or **T**\(_1\)-based [DOX]. Depending on the measurement method used, a different number of slices were averaged for each sample. For both studies, **T**\(_1\)-based [DOX] was the independent variable, and invasive [DOX] was the dependent variable. Thus, the ability of the **T**\(_1\)-based [DOX] measurement to predict the invasive [DOX] was assessed. In particular, we are interested in finding an agreement between invasive [DOX] and **T**\(_1\)-based [DOX], since they are used to measure the same target ([DOX] concentration in vivo). That means we would like to see a regression line of invasive [DOX] vs. **T**\(_1\)-based [DOX] with a slope that is not significantly different from one, and an intercept that is not significantly different from zero, as well as a clinically small measurement error or mean prediction error.

For the HPLC [DOX] samples (punch biopsies) we used only one slice, which was a 3-4-mm slab of tissue. The corresponding **T**\(_1\)-based [DOX] samples averaged the voxels of the ROI over three to four slices, since the MRI slice thickness was 1 mm. For regression of the HPLC [DOX] vs. **T**\(_1\)-based [DOX], we performed the subject-level analysis by averaging all the slices of all the samples for each animal (N = 9). The results were similar to those obtained in the sample level regression analysis and hence are omit-
Alternatively, we performed the sample-level analysis by averaging the slices for each $T_1$-based [DOX] sample that roughly corresponded to the HPLC [DOX] sample and correlated to the HPLC [DOX] sample ($N = 48$). A mixed-effects model was used to take into account the correlation among multiple measurements within each subject. We performed an agreement test to check for statistical differences between the slope and one, and between the intercept and zero. Six samples were excluded from these analyses due to systematic error at [DOX] > 50 ng/mg, as described above.

Fluorescence [DOX] samples averaged the ROIs over at least three slices, with a slice thickness of 15 μm. For the corresponding $T_1$-based [DOX], voxels of similar ROIs were sampled over at least five slices (slice thickness = 1–1.3 mm). Both subject- and sample-level analyses that correlated each $T_1$-based [DOX] measurement to the fluorescence [DOX] measurement were performed, and similar tests were carried out ($N = 10$ and 17, respectively). The results did not differ between the two regions considered (enhancing or non-enhancing) or among the other grouping factors (NTSL or TSL ± HT).

**RESULTS**

A typical animal result is shown in Fig. 1a–f for a TSL with HT experiment. Calculating the DOX concentration began with an initial scan (Fig. 1a), resulting in an initial $T_1$ map (Fig. 1b) calculated by Eq. [1]. The respective liposome drug/HT combination was then administered. A subsequent signal image (Fig. 1c) was obtained (after cooling) and a postcontrast cooled $T_1$ map was calculated (Fig. 1e). (The dynamic results for the different liposome HT combinations are shown in Ref. 4.) Using the difference in pre/post $T_1$ maps/measurements, along with in vitro relaxivity measurements (4), we determined [Mn$^{2+}$] for each voxel using Eq. [1]. We then calculated the DOX concentration using the loading ratio of Mn$^{2+}$:DOX (Fig. 1c, enlarged in f).

The results for both studies were then plotted with $T_1$ calculated [DOX] vs. HPLC or fluorescence measured [DOX] in the range of 0–50 ng/mg (Fig. 1g) and 20 ng/mg (Fig. 1h), respectively. HPLC samples from each animal were plotted to demonstrate the minimal scatter and that in each animal results do not cluster. Additionally, the results show a relationship that is independent of the liposome dosage delivered. When a mixed-effects model was used to account for within-subject correlation, HPLC [DOX] was linearly related to $T_1$-based [DOX] with a slope of 0.86 ± 0.07 (s.e.), and an intercept of −0.01 ± 1.45 ng/mg (Table 1). The slope and intercept were not statistically different from one ($P = 0.09$) and zero ($P = 0.99$), respectively.

A similar agreement between histologic [DOX] and $T_1$-based [DOX] was also linear, with a slope of 0.94 ± 0.13 (s.e.) and an intercept of 0.56 ± 0.72 ng/mg (Table 1). Again, the slope and intercept were not statistically different from one ($P = 0.61$) and zero ($P = 0.45$), respectively. The results show that the relationship between [DOX] measured with HPLC or fluorescence and $T_1$-based [DOX] was independent of the type of liposome carrier used and whether or not HT was administered.

The overlay of both experiments is shown in Fig. 1i, which demonstrates the robustness of this technique in terms of the reproducibility and feasibility of MRI for measuring tissue concentration distributions for this unique drug formulation. Overall, these results indicate a sensitivity that is adequate for evaluating therapeutically relevant levels of DOX using the current MRI technology (3,4).

The sensitivity and error of this measurement technique are dependent on the measured concentration of Mn$^{2+}$ and/or DOX, with error increasing at high concentrations (supplemental data available by request from the corresponding author). One reason for this trend is the breakdown of the assumption that $T_2$ effects can be neglected (see Eq. [1]). At higher contrast concentrations, $T_2$ effects influence the measured concentration by causing excessive decay of the MR signal. Another consequence of high contrast agent concentration is extreme $T_1$ shortening, which causes signal saturation. Therefore, small errors in signal, such as those generated by imperfect image registration, translate into large errors in calculated [DOX]. The extent of error in [DOX] when measured above 50 ng/ml prohibited the inclusion of these ROIs in the HPLC in vivo correlation (Fig. 1g).

A different limitation occurs when the fluorescence intensity is used to calculate the DOX concentration in histological slices above 30 ng/mg. In this case, saturation of fluorescence signal occurred. This was not a limitation for the HPLC method, however. The limitations of the MR method mean that $T_1$-based [DOX] values above 50 ng/mg cannot be measured accurately. This is not a practical limitation, however, because in prior preclinical studies the maximum concentrations of the drug in tumors following TSL+HT, as measured with HPLC, were in the range of 10–30 ng/mg, which is below this threshold. (See supplemental data for further information.)
Another important source of error is the assumption that the Mn$^{2+}$/DOX ratio remains constant after drug release. This does not account for discrepancies in physical properties that can lead to different transport behavior in tissue. DOX is more likely to be retained in the local tissue after release, considering its larger molecular weight and tendency to bind to proteins. Additionally, some Mn$^{2+}$ ions may have washed out of the tumor during the time between the tissue harvesting and the final MRI.

We tested this experimentally by measuring [Mn$^{2+}$] with inductively coupled plasma mass spectrometry for each biopsy sample (in conjunction with DOX) by HPLC. The Mn$^{2+}$/DOX ratio in the tumor samples (0.35 ± 0.23 ng/mg ng/mg, mean ± SD, N = 48) was statistically lower ($P = 0.0007$ by paired $t$-test) and much more variable than the ratio of Mn$^{2+}$/DOX in the injected liposomes (0.42 ± 0.04 ng/mg ng/mg). In these studies, we allowed 90 min of HT/liposomal uptake before the tissues were removed for colocalization analysis. Theoretically, killing the animals earlier would have improved the colocalization of Mn and DOX; however, we chose to observe the full kinetics of uptake and to allow the tumor to cool for the last set of images. The MR images suggest that the released Mn$^{2+}$ was mostly retained within the heated volume, although a portion would likely be present in the efferent vasculature. There was no difference in the Mn:DOX ratio as a function of distance from the center of the tumor (data not shown), which presumably would occur if the colocalization assumption was invalid. Allowing the tumor to return to normothermia reduced the confounding effect of temperature on $T_1$ relaxivity, which we believe would have created even more artifact. Although sources of error existed, they did not significantly limit our ability to use this technique to measure DOX concentration in vivo (Fig. 1, Table 1). Consequently, the results provided a robust validation for the use of MRI to measure drug distribution.

**DISCUSSION**

The results of this study may be applicable to a variety of drug delivery systems. For example, several types of drugs can currently be loaded into liposomes using MnSO$_4$. Examples include the vinca alkaloids (14), other anthracyclines (5), and camptothecins (15), as well as any agent that forms a complex with Mn$^{2+}$ or loads through pH gradients generated and maintained by addition of the ionophore A23187 (14). This method may also be expanded to any drug delivery system that incorporates both a drug and a contrast agent, such as microspheres, peptide polymers, monoclonal antibodies, and cellular-based therapies (12, 16–20).

There is increasing interest in developing ways to tailor and monitor drug delivery in individual patients, particularly cancer patients. For example, pharmacogenomics has been shown to identify subpopulations of patients who show unfavorable drug pharmacokinetics (21–26). The type of imaging described in this paper may provide a method for real-time evaluation of drug delivery that can offer pharmacodynamic information about tumor drug levels, as well as plasma drug levels. This is especially pertinent to preclinical and clinical studies of drug-delivery vehicles and targeted therapies, because such complex drug delivery is inadequately described by plasma studies alone. Overall, this method affords a more rigorous standard for drug dosimetry, which we term “chemodosimetry.”

It has been known for over a century that effective radiation therapy requires precise and uniform dose coverage of the target volume (27). The radiation dose distribution can be calculated because the tissue dependence of energy absorption is known. The development of an analogous chemodosimetry would facilitate the prediction of chemotherapy response on an individual patient basis, as has been achieved with radiation dosimetry. However, this requires methods to measure the drug concentration distribution on a whole-tumor scale (28). Currently, there are few methods that can accurately measure tissue drug concentration distribution in vivo. Imaging methods that have been studied (28–38) include PET, SPECT, CT, and MRI. Direct measurements have been taken using either microdialysis or tissue biopsies. Each of these methods has advantages and disadvantages in terms of quantitative measurement and spatial and dynamic resolution.

PET and SPECT imaging can yield quantitative concentration data for radiolabeled pharmaceuticals. However, spatial resolution is limited. PET radioisotopes require a cyclotron and have a short half-life (29). CT allows excellent spatial/temporal resolution, but it produces ionizing radiation and requires a high-density contrast agent, such as iodine (28). Consequently, the best CT imaging results using liposomes are obtained when imaging in the reticuloendothelial system (30–34, 39). The method described in this report has several advantages. MRI has excellent spatial/temporal resolution, does not use ionizing radiation, and can employ a variety of usable/stable contrast agents. Specifically, loading of liposomes using MnSO$_4$ or other MR contrast agents can be performed in a hospital pharmacy with manufactured reagents and minimal training.

However, the main limitation of MRI is the low SNR that can be encountered with Mn$^{2+}$-labeled drugs (34, 35, 38). The MR sensitivity requires a [Mn$^{2+}$] of at least 0.0175 mM (post-release) in the tumor to obtain adequate signal intensity based on the $T_1$, $S_0$, and relaxivity values from our experiments (4). The corresponding minimum [DOX] would be in the range of 2 ng/mg (see supplemental data). Tumor [DOX] levels were previously reported as 25 ng/mg and 5 ng/mg in vivo after administration of the maximally tolerated dose of DOX containing TSL and NTSL, respectively (3). This demonstrates that MRI has the necessary sensitivity for in vivo measurements using multiple liposome formulations. Improvements in SNR can be obtained if relaxivity is increased beyond that for the free anion. We previously showed that the relaxivity of Mn$^{2+}$ is higher in the presence of lipid (4). When Mn$^{2+}$ is attached to macromolecular drug carriers, the relaxivity may also be higher because of slowed tumbling rates, thereby allowing for greater interaction with water (4, 8).

For clinical development and use, a drug-delivery system and its contents must be well tolerated when administered systemically. The experiments described here were conducted using various DOX doses (7.5–12.5 mg/kg for the HPLC experiments, and 10 mg/kg for the fluorescence
experiments) and different delivery methods (NTSL ± HT, and TSL ± HT for the fluorescence experiments). This study indicates that the relationship between T1,DOX and direct tissue measurements appears to be independent of the liposomal carrier used and whether HT was administered. Additionally, the doses administered never exceeded the maximally tolerated dose of thermosensitive liposomal DOX(3). The corresponding Mn²⁺ dose for the given DOX was 58.7–97.9 μmol/kg, which is 22.5–37.5% of the single dose LD₅₀ (272 μmol/kg) (39).

Additional studies are required to determine the pharmacokinetics and toxicity of circulating Mn²⁺ after drug release. Alternatively, one could use other contrast agents that are less toxic, such as chelated gadolinium. The challenge would be to develop novel methods to load both drug and contrast agent. Once such combinations are achieved, this study provides the foundation for the preclinical development and validation of any imageable liposome.

In summary, we have demonstrated the feasibility of using MRI to measure drug concentration distributions in vivo. Two independent studies validated the finding that T₁,DOX can be used to accurately predict tumor [DOX] in the clinically relevant range of 2–50 ng/mg after liposomal drug delivery. The ability of this method to monitor real-time pharmacokinetics permits evaluation of complex drug delivery systems in vivo. Furthermore, this type of noninvasive “chemodosimetry” could facilitate clinical evaluation of treatment variables and prediction of patient response. Additional validation studies are required to meet this ultimate goal.

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