LABORATORY INVESTIGATION

MR Imaging Enables Measurement of Therapeutic Nanoparticle Uptake in Rat N1-S1 Liver Tumors after Nanoablation

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ABSTRACT

Purpose: To test the hypothesis that magnetic resonance (MR) imaging can quantify intratumoral superparamagnetic iron oxide (SPIO) nanoparticle uptake after nanoablation.

Materials and Methods: SPIO nanoparticles functionalized with doxorubicin were synthesized. N1-S1 hepatomas were successfully induced in 17 Sprague-Dawley rats distributed into three dosage groups. Baseline tumor R2* values (the reciprocal of T2*) were determined using 7-tesla (T) MR imaging. After intravenous injection of SPIO nanoparticles, reversible electroporation (1,300 V/cm, 8 pulses, 100-μs pulse duration) was applied. Imaging of rats was performed to determine tumor R2* values after the procedure, and change in R2* (ΔR2*) was calculated. Inductively coupled plasma mass spectrometry was used to determine intratumoral iron (Fe) concentration after the procedure, which served as a proxy for SPIO nanoparticle uptake. Mean tumor Fe concentration [Fe] and ΔR2* for each subject were assessed for correlation with linear regression, and mean [Fe] for each dosage group was compared with analysis of variance.

Results: ΔR2* significantly correlated with tumor SPIO nanoparticle uptake after nanoablation ($r = 0.50$, $P = .039$). On average, each 0.1-ms^{-1} increase in R2* corresponded to a 0.1394-mM increase in [Fe]. There was no significant difference in mean SPIO nanoparticle uptake among dosage groups ($P = .57$).

Conclusions: Intratumoral SPIO nanoparticle uptake after nanoablation can be successfully quantified noninvasively with 7-T MR imaging. Imaging can be used as a method to estimate localized drug delivery after nanoablation.

ABBREVIATIONS

ΔR2* = change in R2*, DOX = doxorubicin hydrochloride, Fe = iron, HCC = hepatocellular carcinoma, ICP-MS = inductively coupled plasma mass spectrometry, IO = iron oxide, SPIO = superparamagnetic iron oxide

Current chemotherapeutic regimens are limited by systemic toxicity and the inability to quantify delivery of therapeutic agents to the target tumor. Nanoparticles, defined as particles measuring 1–100 nm, are a promising new class of agents that offer several benefits as potential drug delivery vehicles (1,2). Nanoparticles (a) carry a relatively large payload because of their high surface-to-volume ratio, (b) can exploit the enhanced permeability and retention effect, and (c) can be customized with various moieties to serve dual diagnostic and therapeutic purposes (3–5). However, nanoparticle delivery to target tumors has been limited by rapid clearance of nanoparticles by the reticuloendothelial system as well as unpredictable vascular barriers secondary to the heterogeneity of the enhanced permeability and retention effect in large or metastatic tumors (6,7).

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Nanoablation, which combines intravenous nanoparticle delivery with local reversible electroporation, is an innovative method to increase nanoparticle delivery (8). In contrast to irreversible electroporation, which uses intense electric pulses to induce cell death through permanent cell membrane defects, reversible electroporation employs a series of electric pulses that transiently increase the permeability of the targeted cells, resulting in selectively increased drug delivery (9–11). Nanoablation differs from microwave ablation and radiofrequency ablation in that it does not induce necrosis through thermal measures (12). It is a versatile therapy with many applications because it enhances uptake of superparamagnetic iron oxide (SPIO) nanoparticles in both hepatic and nonhepatic tumors compared with standard intravenous dosing (8,13). Additionally, local electroporation can be combined synergistically with selective intraarterial nanoparticle delivery (8).

SPIO nanoparticles act as magnetic resonance (MR) imaging contrast agents because their superparamagnetic core causes more rapid T1 and T2 relaxation of immediately surrounding tissues (14). Gradient echo sequences designed to measure T2* relaxation, defined as the decay of transverse magnetization, are particularly sensitive to the changes induced by SPIO nanoparticles (15). SPIO nanoparticle concentration has been shown to be proportional to the observed change in R2* (ΔR2*), the reciprocal of T2* (14,16,17). However, the quantitative nature of the relationship between intratumoral SPIO nanoparticle concentration and ΔR2* after nanoablation must be established to determine reliably and noninvasively the quantity of chemotherapy delivered to the tumor with this therapy. We tested the hypothesis that MR imaging can be used to predict quantitatively the intratumoral uptake of therapeutic nanoparticles after nanoablation.

MATERIALS AND METHODS

Animal Model

All experiments were approved by the institutional animal care and use committee. Tumors were implanted in 18 male Sprague-Dawley rats (Charles River Laboratories, Wilmington, Massachusetts) weighing 250–380 g. All rats received a standard laboratory diet with free access to water. The N1-S1 rat hepatoma cell line (ATCC, Manassas, Virginia) was obtained and cultured in Dulbecco's Modified Eagle's Medium (ATCC) supplemented with 10% fetal bovine serum (Sigma-Aldrich, St. Louis, Missouri) and 1% penicillin streptomycin (Invitrogen Corp, Carlsbad, California). Cells were maintained in suspension culture flasks at 37°C in a humidified atmosphere containing 5% carbon dioxide. Trypan blue staining was performed before each tumor implantation procedure to verify > 90% cell viability. N1-S1 hepatomas were implanted in the left lateral lobe of the liver and grown for 7–10 days according to a published protocol (18).

Therapeutic Agent and Dosage Groups

Iron oxide (IO) nanoparticles were prepared using iron oxide micropowder as the iron (Fe) precursor, oleic acid as the ligand, and octadecene as the solvent, as described previously (19). The core and hydrodynamic sizes of the IO nanoparticles were measured using transmission electron microscopy and light scattering scan, respectively. IO nanoparticles with 10-nm core size were used for this study. The particles were coated with amphiphilic polymers, reported previously, which stabilize IO nanoparticles in water and provide reactive carboxyl groups on the particle surface for bioconjugation (20). To reduce nonspecific binding and uptake by cells, PEG-diamine (molecular weight, 2,000) was conjugated to IO nanoparticles by the ethyl-3-dimethyl amino propyl carbodiimide coupling method. PEG-diamine was chosen instead of PEG-monoamino because it was thought it would better neutralize the SPIO nanoparticle charge and permit uptake.

Doxorubicin hydrochloride (DOX) in 0.15 mol/L sodium chloride at 0.5 mg/mL was added to the 10-nm IO particles and vortexed for 1 hour at room temperature. DOX was attached to the IO core via a pH-labile bond, allowing it to release DOX selectively in the endosomes and lysosomes of tumor cells after uptake as shown previously (21,22). The free DOX molecules were separated twice from the encapsulated DOX-SPIO nanoparticles by a magnetic separator (SuperMag Separator; Ocean NanoTech, Springdale, Arkansas). The DOX loading amount was 20% (w/w Fe) calculated by free DOX left from the supernatant. These DOX-SPIO nanoparticles were dissolved in deionized water and employed at a concentration of 4 mg/mL Fe.

DOX-SPIO nanoparticle dosages of 0.25 mg/kg, 0.5 mg/kg, and 0.75 mg/kg were used for the three treatment groups (six animals each in the 0.25 mg/kg and 0.50 mg/kg groups and five animals in the 0.75 mg/kg group). These dosages were chosen to approximate clinically relevant DOX concentrations from previous chemoembolization studies (23–25) and to be consistent with previous animal work on nanoablation (8), while attempting to create a range of concentrations for correlation analysis and to investigate a possible dose-response relationship.

MR Imaging

A ClinScan 7-tesla (T) MR imaging horizontal bore scanner (Bruker, Billerica, Massachusetts) with a custom-built rodent receiver coil (Chenguang Medical Technologies Co, Ltd, Shanghai, China) was used for all scans. A mixture of 2%–5% isoflurane and 2 L/min oxygen was supplied via facemask to the subjects during imaging before the procedure. A small animal monitoring system (SA Instruments, Inc, Stony Brook, New York) was used to ensure appropriate sedation and monitor physiologic parameters. Localizer and T2-weighted anatomic scans were performed to verify