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Ultrashort Echo Time (UTE) Imaging of Receptor Targeted Magnetic Iron Oxide Nanoparticles in Mouse Tumor Models

Liya Wang, MD^{1,2}, Xiaodong Zhong, PhD³, Weiping Qian, MD⁴, Jing Huang, PhD^{1,2}, Zehong Cao, MS⁴, Qiqi Yu, MS^{1,2}, Malgorzata Lipowska, PhD¹, Run Lin, MD^{1,2}, Andrew Wang, PhD⁵, Lily Yang, MD, PhD^{4,*}, and Hui Mao, PhD^{1,2,*}

¹Department of Radiology and Imaging Sciences, Emory University School of Medicine, Atlanta, Georgia, USA

²Center for Systems Imaging, Emory University School of Medicine, Atlanta, Georgia, USA

³MR R&D Collaborations, Siemens Healthcare, Atlanta, Georgia, USA

⁴Department of Surgery, Emory University School of Medicine, Atlanta, Georgia, USA

⁵Ocean Nanotech LLC, Springdale, Arkansas, USA

Abstract

Purpose: The purpose of this study was to investigate an ultrashort echo time (UTE) imaging approach for improving the detection of receptor targeted magnetic nanoparticles in cancer xenograft models using positive contrast.

Materials and Methods: Iron oxide nanoparticle (IONP) conjugated with tumor targeting ligands were prepared. A 3D UTE gradient echo sequence with the shortest TE of 0.07 msec was evaluated on a 3T magnetic resonance imaging (MRI) scanner using IONP solution, cancer cells bound with targeted IONPs and orthotopic human pancreatic, and breast cancer mouse models administered tumor targeting IONPs. A simulation was performed to analyze contrast-to-noise ratios (CNR) of UTE images and subtraction of the images obtained UTE and longer TE (SubUTE). T2-weighted imaging and T2 relaxometry mapping were applied for comparison and validation.

Results: UTE and SubUTE images showed positive contrast in pancreatic tumors accumulated with EGFR targeted ScFvEGFR-IONPs and mammary tumors accumulated with uPAR targeted ATF-IONPs. The positive contrast observed in UTE images was consistent with the negative contrast observed in the T2-weighted images. A flip angle of 10° and a maximal possible TE for the second echo are suitable for SubUTE imaging.

Conclusion: UTE imaging is capable of detecting tumor targeted IONPs in vivo with positive contrast in molecular MRI applications.

Keywords

magnetic resonance imaging; magnetic nanoparticle; ultrashort echo time; positive contrast; targeted contrast agent; molecular imaging

MAGNETIC IRON OXIDE NANOPARTICLES (IONPs) have been used as contrast agents of magnetic resonance imaging (MRI) for diagnostic imaging (1-3) and more recently for developing MRI-based molecular imaging probes in various biomedical applications, including cell tracking (4,5), biomarker targeted cancer imaging (6-8), and image guided drug delivery (9-11).

IONPs, as a superparamagnetic agent, primarily shorten transverse relaxation times, ie, T₂ and T₂^{*}, which leads to prominent signal decrease or "negative contrast" of targeted tissue on T₂- and T₂^{*}-weighted images (12-14). The typical drawback of the T₂- and T₂^{*}-weighted negative contrast is its poor sensitivity when used to study areas with low background signal. In addition, T₂- and T₂^{*}-weighted imaging methods are also vulnerable to blooming artifacts and the partial volume errors from the effect of local magnetic inhomogeneity and high susceptibility of paramagnetic IONPs (15,16), making accurate localization and quantitative imaging of magnetic nanoparticles more difficult. It should also be mentioned that further improvement of negative contrast is limited by the threshold of the negative contrast which is the difference between the signal level of IONP affected area and signal intensity of zero (17). Therefore, conventional T₂- and T₂^{*}-weighted imaging methods may not be able to take advantage of those magnetic nanoparticle probes with very high r₂ relaxivity or increase of the dosage of administered IONPs because of the threshold of the negative contrast, as the maximal contrast that the negative contrast can reach is the signal intensity of zero. Therefore, a method of generating positive contrast from magnetic nanoparticles, ie, signal brightening, to improve the detection, and even quantification, of magnetic nanoparticles is desirable and has attracted increasing effort in translating magnetic nanoparticle imaging probes to broader applications (18-20).

A number of approaches have been investigated to obtain positive contrast from IONPs, including ultrashort echo time (UTE) imaging (18,21,22), off-resonance saturation (ORS) techniques (23), sweep imaging with Fourier transformation (SWIFT) (24-27), phase contrast (28-30) and, most recently, adiabatic imaging (31,32). Among them, UTE imaging offers a relatively simple strategy by taking advantage of high longitudinal relaxivity r₁ of IONPs while reducing the contribution of predominant T₂ and T₂^{*} and spin dephase effects of IONPs that are also sources of imaging artifacts. With a very short echo time, typically below 0.1 msec, UTE imaging enables capturing signal enhancement from T₁ effect with little influence of signal decay from the T₂ and T₂^{*} effect, allowing for obtaining positive contrast on T₁-weighted UTE images. Furthermore, the signal enhancement obtained from the UTE imaging is a function of T₁ relaxation times of IONPs at different core sizes and concentrations in a certain range (18). A recent study by Girard et al (22) discussed the general strategy to optimize IONP detection sensitivity using UTE imaging. Through detailed computer simulation, imaging experiments on IONP phantoms in vitro and a proof-of-concept mouse model bearing human prostate tumors in vivo, it was shown that UTE

imaging and subtraction of a longer echo signal from that of the UTE (SubUTE) were good approaches for imaging of IONP.

In the present study, we report further investigation and application of UTE and SubUTE imaging for the detection and visualization of the receptor targeted IONP imaging probes in cancer xenograft mouse models using positive contrast enhancement. The main purpose was to demonstrate the capability of UTE imaging to obtain positive contrast from biomarker targeted magnetic nanoparticle probes selectively accumulated in the human pancreatic and breast cancer xenograft models in the mice *in vivo*.

MATERIALS AND METHODS

Tumor Targeting IONPs and Contrast Properties

Tumor targeted IONPs used in the current study were prepared using methods reported previously (33,34) with a scheme shown in Fig. 1a. A single chain of anti-epidermal growth factor receptor (EGFR) antibody (ScFvEGFR) and an amino terminal fragment (ATF) of urokinase plasminogen activator (uPA) were selected as targeting moieties. Meanwhile, a near-infrared dye, NIR-830, which contains a succinimido carboxylate functional group as an aminoreactive group and was synthesized from 4-mercaptobenzoic acid (Sigma-Aldrich, St. Louis, MO) was conjugated onto IONPs for simultaneous optical imaging as described previously (35).

In order to better understand and optimize the contrast change from UTE images, a simulation analysis similar to that reported by Girard et al (22) was performed. Specifically, the signal intensity S of MR images of a gradient echo (GRE) sequence was simulated for different combinations of TE and flip angle θ using Eq. (1) (36):

$$S \propto e^{-TE/T2^*} \sin\theta \left(1 - e^{-TR/T1}\right) / \left(1 - e^{-TR/T1} \cos\theta\right) \quad [1]$$

In Eq. (1), TR is given by the corresponding experimental values.

For estimating T1 and T2* values of the IONP used in this study, ie, 10 nm core size as shown in the image of transmission electron microscopy (TEM; Fig. 1b), the empirical linear relaxivity approximation described in Eqs. (2) and (3) was used for the simulation at any given IONP concentration (22):

$$\frac{1}{T1} = \frac{1}{T1_0} + r1 [IONP] \quad [2]$$

$$\frac{1}{T2^*} = \frac{1}{T2_0^*} + r2^* [IONP] \quad [3]$$

where $T1_0$ and $T2_0^*$, in the unit of s, are the baseline relaxation times of tissue or solvent without the influence of IONP; $r1$ and $r2^*$ are the longitudinal and transverse IONP relaxivities in the unit of $\text{mM}^{-1}\text{s}^{-1}$; and $[IONP]$ denotes the IONP concentration in mM.

In order to measure r_1 and r_2^* of the IONP used in this study, the baseline $T1_0$ and $T2_0^*$ were first measured from the solvent without IONP. $T1$ and $T2^*$ input parameters for simulation were then obtained from scanning IONP colloidal solutions at five different concentrations, ie, 0.1, 0.12, 0.25, 0.5, and 1 mM, using $T1$ and $T2^*$ mapping sequences as described in a later section. Finally the r_1 and r_2^* relaxivities of the IONP were fitted using Eqs. (2) and (3).

The definitions of contrast (C) and contrast-to-noise ratio (CNR) per unit time in this study were the same as defined in the literature (22). Briefly, contrast is calculated as the signal difference induced by a certain concentration of IONP within a given tissue:

$$C = S_{[IONP]} - S_{[IONP]=0} \quad [4]$$

and CNR is an index of contrast efficiency given by:

$$CNR \propto \frac{C}{\sqrt{TR}} \quad [5]$$

CNR was calculated for both GRE (including UTE) and SubUTE imaging as a function of the paired values of TE and flip angle with Eqs. (1) to (5). In addition, for comparison purposes, we also simulated the CNR of subtraction of the signal at the longer echo from that of the regular GRE imaging with a short TE of 1 msec, ie, SubShortTE contrast, as a function of the paired values of TE and flip angle.

In Vitro Validation of Cancer Cells Targeting With Receptor Targeted IONPs

MiaPaCa-2 is a luciferase-expressing cell line that was derived from a primary orthotopic human pancreatic cancer (37). MiaPaCa-2 cells overexpress EGFR. This cell line was seeded and cultured. After 8-hour incubation (95% air; 5% CO_2 , 37°C) with 10 pmol, single chain fragment of EGFR antibody (ScFvEGFR) conjugated IONPs (ScFvEGFR-IONPs) cells were washed twice with phosphate-buffered saline (PBS) buffer and then detached from the flasks. For testing uPAR targeting, ATF was conjugated on IONPs (ATF-IONP). 4T1CFhR, a luciferase positive mouse mammary tumor cell line with known uPAR overexpression (38), was used with the same experimental conditions as those used for the ScFvEGFR-IONPs.

Specific binding of receptor targeted IONPs to cancer cells was confirmed using confocal microscopy to visualize cellular uptake, Prussian blue staining to visualize iron, and MRI to visualize cells treated with IONPs in cell phantoms. For confocal microscopy experiments, cells were washed with PBS, then fixed with 4% paraformaldehyde (PFA) in PBS for 20 minutes. For Prussian blue staining, each well of the chamber slide was filled with 0.5 mL of 5% potassium ferrocyanide (II) trihydrate and 5% HCl solution and incubated for 2 hours. After being washed three times, cells were counterstained with nuclear fast red for 5 minutes. For MRI scans, cell phantoms were made by mixing cancer cells treated with ScFvEGFR-IONPs with nontreated cells to make a total cell number of 8×10^6 in each. The collected cells were resuspended in 1 mL of 1% agarose gel at 50°C in a glass tube and the

mixture was subsequently cooled to solidify at room temperature. The detailed MRI protocols and parameters are described in later sections. For statistical analysis of correlation between signal intensity of the SubUTE images and amount of the cells bound with ScFvEGFR-IONPs, we performed two-tailed Pearson bivariate correlations in IBM SPSS Statistics v. 20 (Armonk, NY). Outcomes with $P < 0.05$ were considered statistically significant.

Preparation of Orthotopic Human Pancreatic and Breast Cancer Xenograft Models

All animal experiments were performed with the permission of the Division of Animal Resource and Institutional Animal Care and Use Committee (IACUC). Athymic nude mice (Harlan Labs, Indianapolis, IN) bearing orthotopic human pancreatic cancer xenograft tumors ($n = 14$) were prepared by implanting 5×10^6 of MiaPaCa-2 human pancreatic cancer cells in 50 μ L of PBS buffer solution with expression of bioluminescence luciferase into the pancreas of nude mice using a surgical procedure. Orthotopically xenografted pancreatic tumors typically grew to 125 mm³ in 3 to 4 weeks when they were ready for MRI experiments (10). The mouse mammary tumor model was established by injecting 1×10^6 of 4T1CFhR mouse mammary tumor cells in 50 μ L of PBS buffer solution in the mammary fat pad of the female Balb/c mice (Harlan Labs).

Optical Imaging Confirmation of Receptor Targeted IONPs in Tumor Models

Both MiaPaCa-2 human pancreatic cancer cells and 4T1CFhR mouse mammary tumor cells in the current study were luciferase-expressing. The successful tumor implant and tumor growth were monitored by bioluminescence imaging (BLI) weekly and before MRI experiments on tumor-bearing mice using an IVIS in vivo imaging system (Caliper Life Sciences, Waltham, MA).

Near infrared (NIR) optical imaging of the tumorbearing mice was taken via whole body animal imaging using Kodak In vivo Fx imaging system (Carestream Health, New Haven, CT) before and at 24 hours following nanoparticle injection. Tumor-bearing mice were anesthetized by intraperitoneal (i.p.) injection of a ketamine-xylazine mixture. All images were captured using an 800 nm excitation and 850 nm emission filter set with an exposure time of 90 seconds and a γ value of 0.2. For each optical image, a corresponding x-ray radiographic picture was taken to provide the anatomic location of the tumor. The images were analyzed using the Kodak imaging software. After the tumors became palpable, receptor targeted IONPs at 16 nmol/kg of body weight were injected via the tail vein into the tumor-bearing mice. NIR optical and MRI were conducted 24 hours after administering the nanoparticle.

MRI of Phantom and Animal Models

All MRI experiments were performed on a 3 T MR scanner (Tim Trio, Siemens, Erlangen, Germany) using a phased array volumetric wrist coil. T1, T2, and T2* mapping sequences were used to obtain T1, T2, and T2* input parameters for simulation by scanning IONP colloidal solutions at five different concentrations. For T1 mapping, an inversion recovery turbo spin echo (TSE) sequence with a turbo factor of 3, TE of 13 msec, and TR of 1500 msec was used to obtain images at different inversion times (TI) of 23, 46, 92, 184, 368,

650, 850, 1100, and 1400 msec, respectively. For T2 mapping, a multiecho spin echo (SE) sequence was performed with TR of 3000 msec and 20 TEs starting at 12.1 msec with increments of 12.1 msec. In addition, a multiecho 3D GRE sequence was run with TR of 300 msec and 6 TEs starting at 2.90 msec with increments of 3.54 msec for T2* mapping. The 3D GRE sequence, instead of a 2D GRE sequence, was used to reduce the signal void caused by magnetic susceptibility variations in the IONP colloidal solutions (39).

Cell phantoms were scanned using three sequences, ie, a UTE sequence, a T2-weighted TSE sequence, and a T2 mapping sequence. The UTE sequence applied in this study uses GRE acquisition with one 60- μ s long nonselective radiofrequency (RF) pulse, followed by a 40- μ s transmit/receive switch time, and a 100% asymmetric data acquisition from the center to the surface of a sphere in the k -space using a 3D radial sampling trajectory. This UTE sequence was performed in a multiecho fashion, including an ultrashort TE of 0.07 msec and additional four TEs of 3.0, 5.0, 7.0, and 9.0 msec, determined based on the results of simulation and scan time restraint. Other parameters included TR = 11 msec, flip angle = 10° , image matrix = $192 \times 192 \times 192$, field of view (FOV) = $159 \times 159 \times 159 \text{ mm}^3$, leading to an isotropic voxel size = $0.83 \times 0.83 \times 0.83 \text{ mm}^3$, bandwidth = 965 Hz/Pixel, number of readout samples in one radial projection = 384, duration of one radial projection = 883.2 μ s, total radial projections = 42,000, base resolution = 192, and total scan time = 7 minutes 42 seconds. For comparison, the T2-weighted TSE imaging was performed with imaging parameters including turbo factor = 14, TR = 3510 msec, TE = 65 msec, image matrix = 60×256 , FOV = $28 \times 120 \text{ mm}^2$, slice thickness = 1.5 mm and number of averages = 9. In addition, for T2 mapping, a multiecho spin echo (SE) sequence was performed with TR of 3000 msec and 20 TEs starting at 12.1 msec with increments of 12.1 msec, and additional parameters included image matrix = 96×192 , FOV = $64 \times 129 \text{ mm}^2$, slice thickness = 1.4 mm.

For imaging of mouse tumor models, animals were anesthetized by intraperitoneal injection of a ketamine:xylazine mixture (95:5 mg/kg). Mice were placed in the prone position at the center of a volumetric coil with a diameter of 5 cm. The sequences used in the cell phantom studies were applied in animal imaging with selected parameters modified to optimize the SNR, FOV, and resolution. Specifically, TR = 3600 msec, TE = 65 msec, matrix = 128×320 , FOV = $60 \times 120 \text{ mm}^2$, and number of averages = 3 were used in T2-weighted TSE imaging, and TR = 4981 msec, image matrix = 114×192 , FOV = $71 \times 120 \text{ mm}^2$, and slice thickness = 1 mm were used in multiecho SE imaging for T2 mapping.

Image Processing and Data Analysis

MR images were evaluated by measuring signal intensities in the selected regions of interest (ROIs) using ImageJ (National Institutes of Health, Bethesda, MD) (23). ROIs on cell phantoms were manually drawn circles based on the tube size. ROIs of tumors were identified in all slices covering the tumor. ROIs for liver were chosen in three continuous slices in the center of the liver. All ROIs were defined on structural T2-weighted TSE images, and then projected on T2 maps and SubUTE images.

Using an in-house MatLab program (MathWorks, Natick, MA), T1, T2, and T2* values of all pixels were calculated. T1 quantification was done by performing a nonlinear fitting to the signal intensities based on Eq. (6) (22):

$$I = A \cdot |1 - B \cdot e^{-TI/T1}| \quad [6]$$

where A, B, and T1 are free parameters to fit. T2 was fitted based on Eq. (7) (22):

$$I = \sqrt{D^2 + (C \cdot e^{-TE/T2})^2} \quad [7]$$

where C, D, and T2 are free parameters to fit. T2* was extracted by fitting Eq. (8):

$$I = \sqrt{E^2 + (F \cdot e^{-TE/T2^*} \sin \theta (1 - e^{-TR/T1}) / (1 - e^{-TR/T1} \cos \theta))^2} \quad [8]$$

where E, F, and T2* are free parameters to fit.

Since MRI contrast obtained in vivo needs to take into account the background signal level, instead of direct observation of UTE signal from IONPs, one of the alternative strategies to enhance the contrast of targeted tissue or organs was to subtract the signals between ultrashort and long TE so that the signals from background tissues with longer T1 and T2/T2* can be reduced. For UTE imaging, this can be done by subtracting images obtained at longer TEs from the image collected at the ultrashort TE, ie, SubUTE images. This SubUTE approach was demonstrated to enable a synergistic combination of usually antagonist T1 and T2* contrasts (22). In this study, when necessary, the SubUTE images were calculated between the UTE image (0.07 msec) and the images with longer TEs (eg, 3.0, 5.0, 7.0, and 9.0 msec).

Immunostaining and Histochemical Analysis

Mice were sacrificed after the imaging study. Immunochemical staining was performed on the collected tumor and normal tissue samples. Tissue samples were placed into an OCT (optimum cutting temperature) block and immediately frozen in liquid nitrogen. Tissue sections were cut into 5-mm-thick slices. Prussian blue staining was then used to stain the iron oxide nanoparticles. Briefly, the tissue slices were fixed with acetone, incubated with 10% potassium ferrocyanide (II) trihydrate, and 20% HCl solution for 1 hour. After being washed three times, slides were counterstained with nuclear fast red for 5 minutes. The stained slides were examined under a light microscope.

RESULTS

Simulation of UTE and SubUTE signals

The baseline T1₀ and T2₀* of the IONP measured by T1 and T2* mapping sequences are 2.34 and 0.61 s, respectively. Their corresponding r1 and r2* relaxivities are 1.10 and 24.9 mM⁻¹s⁻¹, derived from the data obtained at different IONP concentrations. Fig. 2 F2 demonstrates the simulated CNR evolution of the GRE, including UTE (Fig. 2a,d,g), SubUTE (UTE = 0.07 msec) (Fig. 2b,e,h), and SubShortTE (short TE = 1 msec) imaging (Fig. 2c,f,i) as a function of TE and flip angle at three IONP concentrations. Assuming 0.1

mM IONP as a low concentration in blood or background normal tissue, and 0.5 and 1 mM IONPs accumulated in tumor and liver, respectively, regular GRE imaging may produce positive T1 contrast with normalized CNRs of 0.09 and 0.39 at the tumor tissue IONP concentrations of 0.1 mM (Fig. 2a) and 0.5 mM (Fig. 2d), respectively, using lower flip angles (around 10°) and a short TE (below 2.5 msec in Fig. 2a, and below 0.25 msec in Fig. 2d, respectively). Further reducing TE below 0.15 msec could achieve a normalized CNR of 0.67 at the tumor tissue IONP concentration of 1 mM (Fig. 2g). However, the negative contrast from the T2* effect becomes dominant when lower flip angles (around 5°) and longer TEs are used. For example, the normalized CNR is -0.18 from the tumor tissue IONP concentration of 0.1 mM when TE of 400 msec is applied (Fig. 2a); the normalized CNR reaches -0.38 at the tumor tissue IONP concentration of 0.5 mM when using TE of 200 msec (Fig. 2d); and the normalized CNR further increases to -0.45 at the tumor tissue IONP concentration of 1 mM when using TE of 130 msec (Fig. 2g).

In comparison, SubUTE imaging yields a single positive contrast regime as demonstrated in Fig. 2b,e,h. For the IONP concentrations, ie, 0.1, 0.5, and 1 mM, SubUTE leads to the most pronounced positive CNR of 0.25, 0.69, and 0.99, respectively (Fig. 2b,e,h) compared to CNRs of either positive contrast from T1-weighted UTE imaging (0.09, 0.39, and 0.67) or the negative contrast from T2*-weighted GRE imaging with a regular TE (-0.18 , -0.38 , and -0.45) described above. At the low IONP concentration of 0.1 mM, T2*-weighted GRE imaging with a regular TE (eg, around 400 msec) provides negative contrast CNR of -0.18 , which is higher than the positive contrast provided by T1-weighted UTE imaging for the IONP (0.09). The negative contrast by T2*-weighted GRE imaging with a regular TE (eg, around 200 msec) and the positive contrast from T1-weighted UTE imaging provide the same level of CNR at the IONP concentration of 0.5 mM (0.39 for UTE vs. -0.38 for T2*-weighted GRE imaging with a regular TE). However, when the IONP concentration reaches 1 mM, positive CNR produced by UTE is higher than the negative contrast from T2*-weighted GRE imaging with a regular TE (0.67 for UTE vs. -0.45 for GRE, Fig. 2g). It is noticed that a flip angle around 10° is generally an optimized choice for both UTE imaging and SubUTE imaging as it generates the maximal CNR. Based on the simulation, the maximal possible positive CNRs obtained by SubUTE (0.69 for 0.5 mM in Fig. 2e, 0.99 for 1 mM in Fig. 2h) started to exceed those obtained by SubShortTE slightly (0.68 for 0.5 mM in Fig. 2f, 0.97 for 1 mM in Fig. 2i) with continuous increase at even larger IONP concentrations. Theoretically, the maximal CNR is achieved by SubUTE imaging when the latter TE exceeds 100 msec (Fig. 2b,d,f), although this may not be practical due to the limitation in the length of TR that can be used in a given application. Therefore, the practical and optimized choice of TE for the longer echo in the SubUTE method is usually limited by the allowed TE under the restriction of TR.

Binding and Internalization of the Receptor Targeted IONPs to Cancer Cells

The ScFvEGFR-IONP molecular imaging probe showed effective EGFR targeting with strong binding affinity to MiaPaCa-2 pancreatic cancer cells overexpressing EGFR, evidenced by confocal fluorescent imaging with probes labeled with fluorescent dye (FITC) (Fig. 3a and Prussian blue staining of iron (Fig. 3b). When applying UTE and T2-weighted imaging, cell phantoms containing ScFvEGFR-IONP-treated MiaPaCa-2 cells exhibited

positive contrast enhancement in the SubUTE images, with the samples containing a higher number of IONP bound cells corresponding to the higher intensities (Fig. 3c). In contrast, the signal drop and negative contrast were observed in T2-weighted MRI of the same cell phantoms (Fig. 3d), while T2 relaxation times were reduced when the amount of IONP bound cells increased in the phantoms (Fig. 3e). The levels of the contrast enhancement in the SubUTE images of cell phantoms were consistent with the signal reductions in the corresponding T2-weighted images. Furthermore, the signal intensity of the SubUTE images correlated well ($R^2 = 0.98$, $P = 0.004$), with the amount of the cells bound with ScFvEGFR-IONPs (Fig. 3f), suggesting that increased amount of the cells uptake of IONP contributed to the increased positive contrast in SubUTE images.

UTE Imaging of Receptor Targeted IONPs in Mouse Tumor Models

The ability of obtaining positive contrast from receptor targeted IONP imaging probes in vivo with UTE imaging was tested in two mouse models of pancreatic cancer and breast cancer, respectively. The models are sensitive to the EGFR or uPAR targeted IONP molecular imaging probes as shown in earlier studies (37,38).

Mice with orthotopic human pancreatic tumors grown from MiaPaCa-2 cells, shown as an example in Fig. 4a, were monitored by BLI before MRI experiments. At 24 hours after injection of ScFvEGFR-IONPs tagged with the NIR dye (NIR-830), we observed a strong NIR signal in the tumor (Fig. 4b), confirming the accumulations of ScFvEGFR-IONPs in the tumor. T2-weighted TSE, multiecho T2 relaxometry, and UTE imaging were performed after NIR imaging. When comparing to the images obtained before injection of EGFR targeted ScFvEGFR-IONPs probes (Fig. 4c), the pancreatic tumor showed signal drops in T2-weighted TSE images (Fig. 4d) as the result of the accumulation of ScFvEGFR-IONPs. Correspondingly, SubUTE images showed enhanced positive contrast in the same tumor region (Fig. 4f) compared to the SubUTE images before injection (Fig. 4e), while it is difficult to distinguish positive contrast in the raw UTE images ($TE = 0.07$ msec) obtained before (Fig. 4g) and after (Fig. 4h) IONPs injection. Observed positive contrast was consistent with reduction of T2 relaxation times in tumors with accumulated ScFvEGFR-IONPs (Fig. 4j), compared with those before receiving the contrast agent (Fig. 4i). It should be noted that similar negative and positive contrast effects from IONPs in the T2-weighted and SubUTE images as well as T2 maps were also seen in the liver where nanoparticles are typically trapped by the reticuloendothelial system (RES). The accumulation of EGFR targeted ScFvEGFR-IONPs detected by MRI was further confirmed by the positive Prussian blue staining of the collected tissue sections for the presence of iron. Examination of tumor tissue sections revealed the presence of Prussian blue positive cells in the tumors (Fig. 4k), but not in the normal pancreas (Fig. 4l). Overall, we observed ScFvEGFR-IONPs induced positive contrast from SubUTE images in 12 out of 16 mice bearing pancreatic tumors that showed all three imaging characteristics, ie, positive contrast in UTE images, the signal drop in colocalized T2-weighted images, and reduction of T2 relaxation time in T2 maps. All tumors with positive contrast in SubUTE images were found to contain iron-positive tumor cells in the histological analysis of collected tumor tissue samples. In animals without exhibition of positive contrast in SubUTE imaging, Prussian blue staining for iron was also

negative ($n = 3$). One mouse died before postcontrast MRI 24 hours after receiving ScFvEGFR-IONPs.

The same approach was used to test uPAR targeted UTE imaging of the mouse mammary tumor model grown from with 4T1CFhR breast cancer cells using uPAR targeted ATF-IONP imaging probe tagged with the NIR dye. Similar to the results obtained in the pancreatic cancer mouse model, SubUTE imaging showed the capability of generating positive contrast in vivo in the mammary tumors accumulated with uPAR targeted ATF-IONPs. Signal drop in the tumor was observed in T2-weighted images after intravenous injection of ATF-IONPs (Fig. 5b) compared with those F5 obtained before injection of uPAR targeted IONPs (Fig. 5a). In contrast, bright signal was found in the same tumor in SubUTE images (Fig. 5D) when compared with images collected before administering the IONPs (Fig. 5c). A T2 map also showed decreased T2 values in the tumor 24 hours after IONP injection (Fig. 5f) compared with that obtained before injection (Fig. 5e). Accumulation of uPAR targeted ATF-IONPs in the mammary tumors was further confirmed by NIR optical imaging of the same animals (Fig. 5g) and Prussian blue staining for iron in the collected tumor tissue samples (Fig. 5h).

In addition, a time-dependent increase of positive contrast by UTE imaging as a result of the gradual increase of uPAR targeted ATF-IONPs accumulated in the tumor was also recorded. The plot in Fig. 6a shows the increase of the SubUTE signal intensities in the tumor and liver as a function of the postinjection time at various postinjection timepoints from 3–48 hours, respectively. In contrast, the background signal intensity in reference to the noise had no significant change over the same period of time. The corresponding SubUTE images are shown in Fig. 6b–e. Prussian blue staining from the tumor tissue sections showed positive staining of iron, confirming the accumulation of ATF-IONPs in the tumor tissue (Fig. 6f). It has been documented that the receptor targeted nanoparticles slowly accumulate in the tumor and can eventually be internalized by tumor cells via receptor mediated internalization. This process may take more than 12 hours in the 4T1CFhR breast cancer model studied previously (37,38), allowing for prolonged tumor retention of the imaging probe and maintaining contrast over time (1,37,40). Our observation of a time-dependent increase of positive contrast from UTE imaging is consistent with this notion.

DISCUSSION

The development of imaging methods that are capable of generating positive contrast is driven by the need of enhancing sensitivity of detection of magnetic nanoparticle molecular imaging probes and improving image interpretation in clinical translations. UTE imaging aims to gain positive contrast by using the T1 effect and minimizing the T2 effect of IONPs. Therefore, the UTE imaging approach may provide a simple and alternative method for imaging of IONPs accumulated in the tumors located in the peritoneal cavity, such as pancreatic cancer, where the background signal level is low and T2-weighted imaging may suffer from IONP induced susceptibility artifacts (18). Our results demonstrated that positive contrast from the receptor targeted IONP probes can be obtained in vivo from different tumor models.

Our simulation results showed that T2*-weighted GRE imaging may be more sensitive in detecting IONPs with negative CNR than UTE imaging with positive CNR in low tissue IONP concentrations (eg, below 0.5 mM with selected IONPs and r_2 relaxivity). However, at a moderate IONP concentration, such as 0.5 mM, UTE achieved CNR comparable to that of T2*-weighted GRE imaging. With further increase of the IONP concentration, such as 1 mM, UTE can provide higher CNR than that of T2*-weighted GRE imaging. Therefore, UTE imaging may be especially advantageous over T2*-weighted GRE imaging for imaging of IONP with high concentration. This is particularly important as the imaging artifacts and distortions often occur in T2*-weighted GRE imaging at high iron concentrations. This observation also supports the notion that T2* GRE may reach the maximal negative contrast at high IONP concentrations or IONP with very high relaxivity.

For all the IONP concentrations in the simulation, SubUTE exhibited the highest possible CNR among three positive contrast methods of UTE, SubUTE, and SubShortTE and the conventional negative contrast method of T2*-weighted GRE. Considering the CNR improvement of SubUTE, a dual- or multiecho UTE acquisition would be preferred. The approach with a dual- or multiecho UTE sequence can generate three different and complementary contrasts in one acquisition: an original positive contrast of UTE at an ultrashort TE value, a negative contrast of T2*-weighted GRE at longer TE, and SubUTE with positive contrast that needs to be calculated from the first two sets of images. By subtracting UTE and longer TE images, it also allows for reducing the influence of background signals from tissue/materials with very long T2.

Based on the simulation results, a flip angle of 10° is an optimal choice for both in vivo UTE and SubUTE imaging of the IONP and the concentration range used in this study. As for the selection of longer TE for the SubUTE imaging, due to synergistic T1-T2* characteristics of SubUTE, the theoretical optimal value shown in our simulation was within 100–1000 msec, which is essentially the optimal TE for T2*-weighted gradient echo imaging that gains the maximal sensitivity with negative contrast. However, such a long TE is not practical, because it would require a very long TR and substantial increase of the total scan time. For a TR of 11 msec used in this study, the maximal allowed TE (9 msec) chosen within the restriction of TR provided the optimal CNR for SubUTE. Furthermore, it should be noted that the achievable CNR was largely related to the concentration of the IONP, for which an assumption was made that IONP concentrations of 0.1, 0.5, and 1 mM may represent the IONP concentrations accumulated in the background tissue, tumor, and liver, respectively.

Although SubUTE images were used to suppress the background tissue signal, bright signals can be also found in areas without the presence of IONPs. This is largely attributed to the fact that many short T2 and high susceptibility materials may present in the tissues and organs in vivo. Therefore, it is difficult to select one or several TEs to fully suppress the signal from all the background tissues. In-depth investigation is granted as more sophisticated and selective UTE methods can be developed to address this limitation. Future in vivo applications of UTE methods may be improved by implementation of background signal suppression pulses to the currently used UTE sequences (41). On the other hand, high

T1 or high $r1/r2$ contrast magnetic nanoparticles can be also developed for UTE imaging of specific targeted IONPs to improve tumor detection and diagnosis.

It should be noted that several limitations of UTE imaging should be recognized when applying for imaging of targeted magnetic nanoparticle probes in vivo. First of all, UTE imaging typically requires a relatively long scan time compared to conventional T2-weighted GRE. Furthermore, UTE imaging is advantageous and more sensitive over regular T2 and T2* imaging when IONP concentrations are high in the tissue, but may not be beneficial when the tissue IONP concentration is low. In addition, use of the SubUTE approach requires subtraction of two sets of images. Even though they are acquired at the same scan, the misregistration and motions of the sample/subjects may cause image artifacts and contrast change. We also observed that the positive contrast might arise from other short T2 tissues and materials, which could cause potential artifacts and noises for image interpretation and diagnosis. Future development and in vivo applications of UTE methods need to better differentiate IONPs from other short T2 materials and address the aforementioned limitations.

In conclusion, we demonstrated that UTE imaging can be used for obtaining positive contrast from receptor targeted magnetic nanoparticle molecular imaging probes in orthotopic pancreatic and breast cancer models. UTE imaging not only can potentially improve the sensitivity and specificity of detecting such molecular imaging probes, but also provides an alternative imaging method for facilitating reading and interpretation of images in clinical translation of magnetic nanoparticle imaging probes used for targeted molecular MRI.

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Figure 1. Schematic illustration of synthesis of ATF or SvFcEGFR conjugated IONPs (a), and transmission electron microscopy image of IONP used in the study (b).

Figure 2.

Simulated CNR from IONP solutions as a function of TE and flip angle for GRE, SubUTE (UTE = 0.07 msec), and SubShortTE (TE = 1 msec). Calculations were performed using relaxation times obtained from IONP used in the study at different concentrations with following input parameters: TR = 11 msec, $T1_0 = 2.34$ s, $T2_0^* = 0.61$ s, $r1 = 1.10$ mM⁻¹s⁻¹, $r2^* = 24.89$ mM⁻¹s⁻¹. Isocontours are normalized based on the maximal achievable contrast in these three concentrations (indicated by the 0.99 contour in **h**).

Figure 3.

Fluorescent imaging (a) and Prussian blue staining for the presence of iron (b) confirm the uptake of ScFvEGFR-IONPs labeled with FITC (in green) by pancreatic cancer MiaPaCa-2 cells after cells were treated with ScFvEGFR-conjugated IONPs. Images of cell phantoms containing different numbers of MiaPaCa cells labeled with ScFvEGFR-IONPs were obtained from SubUTE (c), T2-weighted TSE imaging (d), and T2 mapping (e). Increase of positive contrast as a result of the increased number of labeled cell is consistent with the reduction of T2 relaxation time and signal level in T2-weighted imaging. A plot of the signal intensities of SubUTE images (f) measured from the images of phantoms containing 1, 2, 4, 6, and 8×10^6 showed strong correlation between the signal level of SubUTE and MiaPaCa-2 cells bound with ScFvEGFR-IONPs.

Figure 4.

Orthotopic human pancreatic tumors implanted in the pancreas grew progressively and were confirmed by bioluminescence imaging (a). Optical imaging showed a strong NIR signal in the tumor mass 24 hours after the administration of NIR dye labeled nanoparticles (b). Pre-contrast T2-weighted image (c), SubUTE image (e), raw UTE image (g), and T2 map (i) were obtained from the animal. The corresponding postcontrast images showed dropped signal intensity in the tumor (arrows and dotted contours) and the liver (yellow solid contours) in the T2-weighted image (d), increased signal intensity in SubUTE images (f) and raw UTE (h), and decreased T2 values in the T2 map (j). Prussian blue staining of frozen tissue sections showed the presence of iron-positive cells, bound and internalized with EGFR targeted IONPs, in pancreatic tumor (k), but not in normal pancreas (l). Interestingly, a high level of accumulation of IONPs in the liver caused signal drop in raw UTE images due to the T2 effect. However, positive contrast can still be obtained with the SubUTE method.

Figure 5. Signal changes in T2-weighted images (**a,b**), SubUTE images (**c,d**) and T2 maps (**e,f**) before (**a,c,e**) and after uPAR targeted ATF-IONPs injection (**b,d,f**) were observed in the tumor of the mouse mammary tumor model. The dotted color contours indicated the tumor location. **g,h**: NIR imaging and pathological data that confirmed the accumulation of IONPs in the tumor.

Figure 6.

Dynamic changes of the positive contrasts in breast tumors after systemic delivery of uPAR targeted ATF-IONPs. There is a strong positive relationship between the postinjection timepoints and the signal intensity in the SubUTE images from tumor and liver where the IONPs accumulated (a). **b–e**: The tumor was outlined with red contours, and the liver is outlined with yellow contours. There was no preinjection-positive signal in the tumor (b). However, a small region with positive signal in the tumor was observed 3 hours post-IONP injection, and much brighter signals were observed in the tumor with a larger region at 24 hours postinjection (d). Finally, the maximized region in the tumor showed the brightest signal intensity at 48 hours postinjection (e). Prussian blue staining showed iron-positive cells in tumor tissue sections obtained from the same mouse mammary breast cancer xenografts.