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Quantitative Contrast-Enhanced MRI with Superparamagnetic Nanoparticles Using Ultrashort Time-to-Echo Pulse Sequences

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Abstract

Purpose: Conventional MRI using contrast agents is semi-quantitative because it is inherently sensitive to extravascular susceptibility artifacts, field inhomogeneity, partial voluming, perivascular effects, and motion/flow artifacts. Herein we demonstrate a quantitative contrast-enhanced MRI technique using ultrashort time-to-echo pulse sequences for measuring clinically relevant concentrations of ferumoxytol, a superparamagnetic iron oxide nanoparticle contrast agent with high sensitivity and precision in vitro and in vivo.

Methods: The method achieves robust, reproducible results by using rapid signal acquisition at ultrashort time-to-echo (UTE) to produce positive contrast images with pure T_1 weighting and little T_2^* decay. The spoiled gradient echo equation is used to transform UTE intensities directly into concentration using experimentally determined relaxivity constants and image acquisition parameters.

Results: A multiparametric optimization of acquisition parameters revealed an optimal zone capable of producing high-fidelity measurements. Clinically relevant intravascular concentrations of ferumoxytol were measured longitudinally in mice with high sensitivity and precision (~7.1% error). MRI measurements were independently validated by elemental iron analysis of sequential blood draws. Automated segmentation of ferumoxytol concentration yielded high quality three-dimensional images for visualization of perfusion.

Conclusions: This ability to longitudinally quantify blood pool CA concentration is unique to quantitative UTE contrast-enhanced (QUTE-CE) MRI and makes QUTE-CE MRI competitive with nuclear imaging.

Keywords

quantification; ultrashort TE (UTE); ferumoxytol; superparamagnetic iron oxide nanoparticle (SPION)

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Additional Supporting Information may be found in the online version of this article.

INTRODUCTION

The ability to measure structural and functional features of health and disease is limited by our current clinical imaging toolbox. Advances in patient monitoring and follow-up need to be quantitative, objective, specific, sensitive, reproducible, and safe (1–3). Currently, only nuclear medicine provides an effective means of quantifying contrast agents (CAs) in vivo (4). However, the radioisotopes involved in these procedures are hazardous, thus the use of nuclear medicine is not warranted for repeat structural and functional imaging (5). Clinical implementation of alternative quantitative imaging techniques could revolutionize patient care.

MRI provides information about blood and tissue without the use of harmful radiation. Over the last decade, many researchers have attempted to collect and analyze MRI data in a quantifiable manner (6). MRI, however, remains semiquantitative because it is inherently sensitive to extravascular susceptibility artifacts, field inhomogeneity, partial voluming, perivascular effects, and motion/flow artifacts (7). Imaging techniques that employ a time-to-echo (TE) of half a millisecond or more are particularly susceptible to heterogeneous signal modifications and are therefore difficult to interpret (8). Despite the relationship between MRI signal intensity and CA concentration being widely recognized to be complex and nonlinear (8,9), current models for contrast CA quantification assume a linear relationship between signal intensity and CA concentration or a linear relationship between CA concentration and relaxation rate (10–13). The error for these models varies from 15% to 30% (Table 1), which is attributed to complicated in vivo signal artifacts, such as flow, organ movement, and susceptibility changes. Given the current limitations in absolutely quantitative MRI, new acquisition and analysis methods are greatly needed.

We report a quantitative contrast-enhanced MRI technique that uses an ultrashort time-to-echo (UTE) to generate positive-contrast images of superparamagnetic iron oxide nanoparticles (SPIONs) in vivo. Ultrafast (e.g., 10–200 μ s) signal acquisition has the benefit of producing positive contrast images instead of dark contrast images by acquiring signal before tissue magnetization in the transverse plane dephases (14), thus allowing complete T_1 contrast enhancement from SPIONs (15). We hypothesize that UTE is uniquely suited for measuring the concentration from clinically relevant concentrations of FDA-approved ferumoxytol. Here, we demonstrate that quantitative UTE contrast-enhanced (QUTE-CE) MRI is a robust technique for quantifying blood pool ferumoxytol in vitro error 3.0% and in vivo 7.1% error in the clinical range. The image acquisition method is anchored in theory and is compatible with existing clinical SPION formulations and scanners, thus QUTECE MRI shows high potential as a quantitative imaging method that is immediately applicable to human scans.

THEORY

The image intensity in a given voxel measured by QUTE-CE MRI is a function of both image acquisition and material parameters:

$$I = f(TE, TR, \theta; T_1, T_2^*, K, \rho)$$

where TE is the time-to-echo, TR is the repetition time, and θ is the flip angle. TE, TR, and θ are image acquisition parameters defined by the user. T_1 and T_2^* are the longitudinal and transverse relaxation times, respectively, that depend on the medium under investigation and the magnetic field strength. K is the constant that relates the UTE signal intensity as seen by the coil and ρ is the proton density of the medium. For ultrashort TE values, T_2^* effectively equals T_2 .

T_1 and T_2 can be written in terms of their reciprocals, called relaxation rates R_1 and R_2 , respectively, for the facile determination of relaxivity constants. For imaging at a single magnetic field strength (7T in this study), the explicit field dependence is omitted. Here, the medium under investigation is ferumoxytol (Feraheme, AMAG Pharmaceuticals, Waltham, Massachusetts, USA) uniformly mixed in blood. Thus, R_1 and R_2 are a function of the initial relaxation rate of the blood (R_{1_0} and R_{2_0}), the longitudinal and transverse relaxivities (r_1 and r_2), and ferumoxytol at given concentrations C .

For concentrations in which the relaxation rate is linear,

$$R_1 = R_{1_0} + r_1 \cdot C \quad [1]$$

$$R_2 = R_{2_0} + r_2 \cdot C \quad [2]$$

Under these approximations, the UTE signal intensity can be approximated by the spoiled gradient echo (SPGR) equation (16)

$$I = K\rho \cdot e^{-TE \cdot R_2} \cdot \sin \theta \cdot \frac{1 - e^{-TR \cdot R_1}}{1 - e^{-TR \cdot R_1 \cdot \cos \theta}} \quad [3]$$

$$I = K\rho \cdot e^{-TE \cdot (R_{2_0} + r_2 \cdot C)} \cdot \sin \theta \cdot \frac{1 - e^{-TR \cdot (R_{1_0} + r_1 \cdot C)}}{1 - e^{-TR \cdot (R_{1_0} + r_1 \cdot C) \cdot \cos \theta}} \quad [4]$$

Once the relaxivity constants have been obtained, the image acquisition parameters have been established, and $K\rho$ has been calibrated, unknown SPION concentrations can be

quantified experimentally using Equation [4]. This procedure was validated both in vitro and in vivo using the techniques described below.

METHODS

QUTE-CE uses CA-induced T_1 shortening, combined with rapid signal acquisition at UTEs, to minimize T_2^* decay. The procedure is summarized in Figure 1. The relevant steps are as follows:

1. Calibration phantoms containing blood (1% heparin) are doped with clinically relevant concentrations of ferumoxytol (0–150 $\mu\text{g/mL}$);
2. for each calibration sample, T_1 and T_2 are measured, from which R_{1_0} , r_1 , R_{2_0} , and r_2 can be extrapolated (Supplementary Fig. 1);
3. a UTE protocol is established with optimized TE, TR, and θ image acquisition parameters and a fixed trajectory (17,18), precalculated with a symmetric phantom;
4. K is measured together with ρ as $K\rho$, assuming the proton density of whole blood is constant, and serves as a calibration for the given UTE protocol;
5. positive-contrast images using the optimized parameters are acquired in vivo; and
6. CA concentrations in each voxel are calculated directly from UTE signal intensity by application of the theory described below.

Characterization of Ferumoxytol Relaxivity in Blood

MRI images were obtained at ambient temperature ($\sim 25^\circ\text{C}$) using a Bruker Biospec 7.0T/20-cm USR horizontal magnet (Bruker, Billerica, Massachusetts, USA) and a 20-G/cm magnetic field gradient insert (ID = 12 cm) capable of a 120- μs rise time (Bruker).

1% whole heparinized male Swiss Webster mouse blood (BioChemed Services, Winchester, Virginia, USA) and calf blood (Lampire Biological Laboratories, Ottsville, Pennsylvania, USA) was stored at 4°C for 0–11 days until experiments.

R_{1_0} and r_1 were measured with a variable TR spin-echo sequence and R_{2_0} and r_2 were measured with a multiecho spin-echo sequence with TR fixed. ParaVision 5.1 software was used to draw regions of interest (ROIs) and calculate relaxation rate values. Decay curves were fit with a monoexponential decay equation to calculate T_2 , $y = A + C_1 e^{-TE/T_2}$, and a monoexponential growth saturation recovery equation to calculate T_1 , $y = A + C_2 \left(1 - e^{-TE/T_2} \right)$, where A is the absolute bias, C_1 is the signal intensity at TE = 0, and C_2 is the signal intensity at TR = ∞ (Supplementary Fig. 1, Supplementary Table 1). Three separate experiments were performed and the relaxivities were averaged to reduce statistical

error. In one experiment, 2-mL ferumoxytol-doped blood phantoms were scanned one at a time (mouse blood), whereas in two other experiments, six ferumoxytol doped phantoms filled with calf blood (0–250 $\mu\text{g/mL}$ ferumoxytol) were arranged in pentagonal fashion, with the 0 $\mu\text{g/mL}$ vial at the center. Only measurements from 0–150 $\mu\text{g/mL}$ ferumoxytol were used to calculate relaxivity to remain in the linear relaxation rate regime. For T_1 measurements, the image acquisition parameters were as follows: field of view (FOV) $5 \times 5 \text{ cm}^2$; 2 mm slice thickness, $\text{TE} = 4.2 \text{ ms}$; $\text{TR} = 100\text{--}2500 \text{ ms}$; rare factor of 1; 17 min, 26s scan time. No saturation pulse was used, and the maximum TR of 2500 ms was greater than $5 T_1$ for all doped blood concentrations. For T_2 measurements, the image acquisition parameters were as follows: $\text{FOV} = 5 \times 5 \text{ cm}^2$; 3 mm slice thickness; $\text{TR} = 1500 \text{ ms}$; $\text{TE} = 4.2\text{--}133.2 \text{ ms}$ (30 echoes); 4 min, 34 s scan time. The large range of TE values was needed to accommodate all concentrations present in the imaging space. Decay and growth curve fits can be found in Supplementary Figures 2, 3, and 4. For mouse blood, ferumoxytol-doped phantoms were placed inside the coil individually to reduce noise, and TE and TR values were adjusted for each concentration range individually for optimal curve fitting. All relaxivity measurements were made inside a 72-mm Bruker quad coil.

The relaxivity of calf blood was determined to be comparable to that of mouse blood, and all calculations using relaxivity constants were performed using the average fittings from the three separate measurements: $R_{10} = 0.85 \text{ s}^{-1}$, $r_1 = 2.12 \text{ mM}^{-1}\cdot\text{s}^{-1}$, $R_{20} = 53.22 \text{ s}^{-1}$, and $r_2 = 33.15 \text{ mM}^{-1}\cdot\text{s}^{-1}$ (Supplementary Fig. 1, Supplementary Table 1) [conversion factor: concentration iron (in mM) = concentration iron (in $\mu\text{g/mL}$)/55.845]. For comparison, the longitudinal relaxivity was similar to one reported in the literature at 7.1T of $2 \text{ mM}^{-1} \text{ s}^{-1}$, albeit for water (19). The r_2 for water was $95 \text{ mM}^{-1}\cdot\text{s}^{-1}$, the difference attributed here as either influenced by the media under study (blood versus water) or inaccuracies in the multiecho spin-echo measurements. Nevertheless, T_2/T_2^* decay for the experiments made herein would be negligible; even at a ferumoxytol concentration as high as $150 \mu\text{g/mL}$, an r_2 of $100 \text{ mM}^{-1}\cdot\text{s}^{-1}$ would only amount to a 0.29% decay of signal from the SPGR equation at $\text{TE} = 120 \mu\text{s}$ (the high range for the optimization experiment) or a 0.03% decay at $\text{TE} = 13 \mu\text{s}$ (the value used for in vitro and in vivo experiments); consequently, inaccuracies in this measurement played a negligible role in our calculations.

$K\rho$ Calibration

Unlike the four relaxivity constants in Equation [4], which only need to be measured for each magnetic field strength, $K\rho$ is a constant that needs to be determined for each imaging protocol, as it depends on acquisition parameters (TE, TR, θ , matrix size) and coil hardware. Thereafter, $K\rho$ can be used for all subsequent scans. Calibrating $K\rho$ is straightforward and was executed using the following steps:

1. Ferumoxytol-doped blood was prepared at known concentrations.
2. A UTE protocol with specific acquisition parameters was performed using the phantoms as prepared from Step 1.

3. ROIs were drawn on the images inside the vials in the center z-axis axial slice of the three-dimensional (3D) image to obtain a mean intensity and standard deviation.
4. The intensity from Step 3 was used in conjunction with the SPGR equation to determine $K\rho$ (TE, TR, θ , C are known parameters, and relaxivity constants were measured in a previous experiment).
5. The average value of $K\rho$ was taken as a calibration constant.

Once this procedure was completed, $K\rho$ was used for all subsequent quantitative calculations using this protocol.

In Vivo Experimental Procedure

All animal experiments were conducted in accordance with the Northeastern University Division of Laboratory Animal Medicine and Institutional Animal Care and Use Committee. The same quadrature 300 MHz, 30 mm Mouse MRI coil was used for all in vivo work (Animal Imaging Research, LLC, Holden, Massachusetts, USA). Healthy anesthetized Swiss Webster mice ($n = 5$) received a one-time intravenous bolus injection of 0.4–0.8 mg ferumoxytol for a starting blood pool concentration of 100–200 $\mu\text{g/mL}$ (diluted to 4 mg/mL in phosphate-buffered saline) and were imaged longitudinally after injection (0, 2, and 4 h). Precontrast images were also acquired. Given the assumption that blood in mice is about 7% of body weight, for a 50-g mouse an initial yield of 115–230 $\mu\text{g/mL}$ was predicted. This is similar to clinical concentrations where an injection of 510 mg produces a blood concentration of about 100 $\mu\text{g/mL}$ for a total blood volume in the average adult human of 5 L.

A single UTE protocol was used for all images. To establish the UTE protocol, the following parameters were fixed: FOV = $3 \times 3 \times 3 \text{ cm}^3$; matrix mesh size = $200 \times 200 \times 200$; TE = 13 μs ; TR = 4 ms; and $\theta = 20^\circ$. TR was slightly higher than the optimal value because of hardware and memory constraints. We analyzed a 50-mL cylindrical phantom filled with 5 mM CuSO_4 to determine the k-trajectories for image reconstruction.

Image Processing

Reconstructed 3D intensity image data were rescaled back to the original intensity measurement (as necessary with Bruker file format files, one must divide by the receiver gain and multiply by scaling factor called SLOPE). Intensity data were then converted to concentration via theory using a custom MATLAB (MathWorks, Natick, Massachusetts, USA) script to solve numerically the nonlinear SPGR intensity in Equation [4]. The signal-to-noise ratio (SNR) is defined as the average signal from an ROI divided by the standard deviation of the noise, as measured in an ROI located in air outside the sample. For the contrast-to-noise ratio (CNR) in phantoms, the mean signal from undoped blood intensity was subtracted from the measurement made in doped blood; for in vivo measurements, tissue surrounding the vasculature was used for contrast. The time-adjusted SNR and CNR take into account the duration of the scan by dividing by $\sqrt{\text{TR}}$, which normalizes SNR and CNR by the scan time.

Autosegmentation Using 3DSlicer

3D autosegmentation was rendered with 3DSlicer (<http://www.slicer.org>) (20) using established modules. First, all voxels within the range of the mean concentration ± 2.5 standard deviations as measured in the left ventricle were selected (ThresholdEffect module). The GrowCut algorithm module was then used to separate out the vasculature from the rest of the image. The ChangeLabel Effect module was used to uniquely select the vasculature segment, for which a model was created with the Model Maker module.

Inductively Coupled Plasma Atomic Emission Spectroscopy

Inductively coupled plasma atomic emission spectroscopy (ICP-AES) was performed to analyze the iron-oxide nanoparticle (IONP) content in doped whole animal blood. Briefly, preparation of IONP-doped media involved the full digestion of the sample in a Milestone Ethos Plus Microwave. Full digestion was achieved by taking 0.1 mL of sample and adding 6 mL of concentrated nitric acid, 2 mL of hydrogen peroxide, and 2 mL of pure water and running a protocol on the microwave that ramped the temperature up to 210°C for 15 minutes. Following digestion, the samples were dried, resuspended in 5 mL of 2% nitric acid, and measured using ICP-AES. A standard curve using a monoelemental iron was run to ensure high instrument fidelity ($r^2 = 1.000$). Each data set ($n = 5$) was fitted with the pooled slope and average intercept ($n = 3$ per set) to account for offsets in baseline iron content, for a total of $n = 15$ in vivo measurements.

RESULTS

3D UTE-CE Imaging with Ferumoxytol

3D UTE-CE imaging with ferumoxytol produced unique images, as most organs that are completely invisible without SPIONs contrast become visible with SPIONs contrast (Fig. 2a,c). Precontrast signal from blood entering the periphery of the image space into the stomach was apparent because of incoming water protons with fresh longitudinal magnetization compared with those that had already been saturated. Postcontrast images rendered high CNR images of all the vasculature in which nanoparticle iron circulated (Fig. 2b,c). Thus, UTE allowed for completely T_1 -weighted snapshot images of CA distributed in vivo. This is atypical in MRI, in which contrast is usually added or subtracted from already apparent regions, and more like nuclear imaging techniques, from which image contrast is solely dependent on CA location and concentration.

100 UTE Experiments Reveal an Optimal Zone at 7T

The ability to predict CA concentrations from UTE intensity using the SPGR equation is influenced by image acquisition parameters TE, TR, and θ . A 3D UTE radial k-space sequence (readily available from the Bruker tool-box) was selected, and the following imaging protocol was established: FOV = $3 \times 3 \times 3 \text{ cm}^3$; matrix mesh size = $128 \times 128 \times 128$; and 51,360 radials, which rendered 234 mm x-y-z resolution images with a 3-min scan time for TR = 3.5 ms. The image reconstruction trajectory was fixed using a 5-mM copper sulfate (CuSO_4) phantom constructed from a 50-mL centrifuge tube. Experiments were performed on whole calf and mouse blood (1% heparin) doped with ferumoxytol (0–250 $\mu\text{g}/$

mL). We used a high bandwidth radiofrequency pulse to avoid complications for cases in which a low bandwidth compared with T_2^* may cause a curved trajectory for the magnetization vector M_z out of the z-plane (21). Assuming $T_2^* \approx T_2$ at UTE values, the 200 kHz bandwidth yielded ultrafast excitation compared with the lowest T_2 value of 5.5 ms at 150 $\mu\text{g/mL}$. All experiments performed on acquisition parameters optimization were performed with a 72-mm Bruker quad coil.

For calf blood, 100 scans were executed covering combinations of five TEs (13, 30, 60, 90, and 120 μs), five TRs (3.5, 5, 7, 9, and 11 ms), and four θ s (10°, 15°, 20°, and 25°). Six 2-mL phantoms of ferumoxytol-doped calf blood at (0–250 $\mu\text{g/mL}$ ferumoxytol) were arranged in pentagonal fashion with the 0 $\mu\text{g/mL}$ vial at the center inside of a 72-mm Bruker quad coil. $K\rho$ was calibrated per image, with the 0 concentration exceptionally excluded in calculations because the noise from surrounding high concentrations rendered a poor measurement. It was found that higher concentration UTE signals deviated from their optimal values from the SPGR equation, owing to the nonlinear behavior of the relaxation rate at high concentrations, thus only 0, 50, 100 and 150 $\mu\text{g/mL}$ phantoms were considered in the analysis in Figure 3. The results are thus relevant for clinical concentrations of ferumoxytol, considering 100 $\mu\text{g/mL}$ is roughly equivalent to a single intravenous bolus of 510 mg in adult humans. Accuracy was observed to be most stable at TE = 13 μs , TR = 3.5 ms, and $\theta = 20^\circ$ (Fig. 3a). In this optimal zone, the average in vitro error between QUTE-CE measurements and known ferumoxytol concentrations was <4 $\mu\text{g/mL}$, but increased significantly as TR and θ deviated (Fig. 3b). However, changes in TE up to 120 μs had little impact on concentration measurements (Fig. 3c). This information is crucial for obtaining precise concentration measurements from theory, and to our knowledge represents the first such account in the literature. The agreement between the measured signal intensity and the SPGR equation for known concentrations at the optimized parameters was excellent (Fig. 3d). The absolute values for SNR and CNR at 150 $\mu\text{g/mL}$ ferumoxytol in the optimal zone were 72 and 57, respectively (Fig. 3e). The time-corrected SNR and CNR also tended to be higher in the optimal zone (Supplementary Fig. 5). Relaxation rate measurements were repeated after the experiment to ensure that no blood coagulation was present (Supplementary Fig. 1). These results validate the use of the SPGR equation (Eq. [4]) to determine unknown concentrations and are the basis of QUTE-CE MRI.

To ensure validity of phantom measurements, experiments were repeated with mouse blood with five TEs (14, 30, 60, 90, and 120 μs) and five TRs (4, 5, 7, 9, and 11 ms) at $\theta = 20^\circ$ (Supplementary Fig. 6). Six 2-mL vials of ferumoxytol (50, 75, 100, 125, 150, and 175 $\mu\text{g/mL}$) were arranged around a center vial of 5 mM CuSO_4 . The same pattern for the optimal zone was confirmed in mouse blood, with absolute concentration errors similar to the previous experiment.

QUTE-CE Calibration and Validation for Mouse Imaging

The same imaging protocol and coil was used as for in vivo experiments (see Methods). Phantoms (0–150 $\mu\text{g/mL}$ ferumoxytol) were placed one at a time for calibration of $K\rho$ to produce ideal images with low noise (Fig. 4a). This protocol and calibration was used for all subsequent in vitro and in vivo experiments.

To assess in vitro performance of QUTE-CE MRI, doped phantoms were created by serial dilution of ferumoxytol from 128 and 96 $\mu\text{g/mL}$. 3D UTE was performed and concentrations were calculated voxel by voxel for images containing multiple phantoms (Supplementary Fig. 7). A linear correlation ($R^2 = 0.998$) was observed between the measured and known ferumoxytol concentrations (Fig. 4b). The average residual error in measured concentration was found to be $2.57 \pm 1.34 \mu\text{g/mL}$, or 3.04% for samples between 48 and 128 $\mu\text{g/mL}$ (Fig. 3b, insert). Measurements were taken at the center of the z-axis in the imaging space, after converting from UTE intensity to concentration (Fig. 4c,d), to minimize inhomogeneous effects from imperfect transmit field (B_1^+) and receive (B_1^-) homogeneity. We assessed the effect of B_1 inhomogeneity on concentration measurements as a function of distance deviated from the center z-axis along the tubular phantoms as far as possible in the 3D images (Fig. 4e, Supplementary Fig. 8). B_1 inhomogeneity was most significant for the highest concentrations, adding about 10% error to the 128 $\mu\text{g/mL}$ phantom at a distance of 50 mm.

Quantification of Blood Pool Ferumoxytol In Vivo

Comparison of the precontrast (Fig. 5a) and postcontrast (Fig. 5b) images showed positive-contrast enhancement, facilitating clear delineation of the mouse vasculature with a comparable SNR (23.2–49.4) and CNR (4.0–41.5) to similar ferumoxytol concentrations in vitro. 3-D segmentation with 3DSlicer, centered the measured mean concentration ± 2.5 standard deviations, allowed reconstruction of numerous vessels (Fig. 5c). To quantify ferumoxytol concentration in blood, blood (200 μL) was drawn after each imaging session and its iron content was measured by ICP-AES analysis (Fig. 5d, Supplementary Fig. 9). QUTE-CE proved to be highly accurate, with an average of 7.07% ($6.01 \pm 4.93 \mu\text{g/mL}$) error across all 15 measurements (concentrations 30–160 $\mu\text{g/mL}$). The maximum observed residual error in vivo was 13.50 $\mu\text{g/mL}$, compared to 5.0 $\mu\text{g/mL}$ in vitro (Fig. 5d, inset). The linear correlation coefficient between ICP-AES and QUTE-CE measurements was $R^2 = 0.954$. The QUTE-CE ROI for quantification was routinely drawn in left ventricle throughout several slices (Fig. 5d, insert) and analyzed in a blinded manner. Almost all ROIs were within 5 mm of the center of the z-axis, which minimized error from B_1 inhomogeneity; thus there was no correlation between error and distance to the center of the z-axis. Longitudinal measurements of ferumoxytol concentration in vivo showed a clear, reproducible decay in blood pool concentration, making it possible to measure the half-life of the CA from images alone. The ferumoxytol half-life was found to be $3.92 \pm 0.45 \text{ h}$, with an average $R^2 = 0.988$ across five mice (Fig. 5e).

DISCUSSION

Ferumoxytol and other SPIONs are generally considered poor candidates for quantitative imaging due to their high T_2^* relaxivity, which results in negative-contrast imaging (22). However, the biocompatibility of SPIONs makes them an attractive candidate for nanoparticle contrast-enhanced MRI (23–25), and even as an alternative to the widely used gadolinium contrast agents, which is now recognized to result in nephrotoxicity, particularly for renally impaired patients (26,27). Quantification of SPIONs using ultrafast acquisition with UTE or SWIFT pulse sequences is currently under investigation (28,29). Our use of the

UTE pulse sequence, which allows us to acquire a signal before the transverse magnetization is dephased (14), takes advantage of the r_1 relaxivity of SPIONs. The ferumoxytol concentration is directly extrapolated from measured signal intensity by using the SPGR equation, which describes the signal evolution and holds true under the optimized conditions studied here.

Published methods to quantify CA concentration (Table 1) generally rely on the linear relationship between measured signal intensity or R_1 relaxation rate and concentration. There still remains a high degree of error with this approach in vivo, reported on the order of 15%–30% (11–13), due to heterogeneous, nonlinear signal changes that are not adequately described by theory when measuring in vivo. Complex nonlinear modeling has shown limited success (9,30,31) ($13\% \pm 9\%$ error in vivo) but is sensitive to magnetic susceptibility, imperfect B_0 shimming, and chemical shifting, all of which worsen at longer TEs and are thus avoided in QUTE-CE MRI. More recently, researchers have attempted to distinguish between CA and tissue signals using a custom-built coil to introduce a time-dependent magnetic field; however, no in vivo results have been reported (32).

Our ability to achieve robust, reproducible results is primarily physical and is contingent on multiparametric optimization of TE, TR, and θ . By choosing optimized image acquisition parameters to minimize the error in concentration, including UTE, we were able to use the SPGR equation to accurately measure ferumoxytol concentrations in vitro and in vivo. This optimized UTE protocol allows signals to be acquired microseconds after excitation, before cross-talk between voxels can occur, thereby eliminating both extravascular susceptibility and flow effects. Indeed, the average blood flow velocity in mice is ~ 10 – 100 mm/s (excluding the largest arteries) (33), thus blood displacement is two orders of magnitude less than the voxel size during image acquisition. A low TR suppresses flow effects for concentration quantification as well as suppressing precontrast tissue signal, rendering high SNR and CNR ratios similar to those observed in vitro. Importantly, this optimization of the UTE protocol yields a strong correlation between the theory and experimental measurements, allowing the QUTE-CE image contrast to be quantified with a precision two to four times that of other reported techniques.

Longitudinal QUTE-CE measurements can be used to determine pharmacokinetic parameters. We have demonstrated the ability to distinguish time-dependent changes in blood pool ferumoxytol concentration with a precision of ~ 0.1 mM at 7T up to ~ 3 M for the estimation of CA half-life. These measurements were independently validated ex vivo using ICP-AES. The ferumoxytol half-life measured in mice by QUTE-CE MRI (3.92 ± 0.45 h) is comparable to that measured by others using radiolabeled ferumoxytol in rats (3.9 h) and rabbits (4.4 h) (34). Importantly, QUTE-CE concentration measurements are extrapolated directly from UTE signal intensities, without pharmacokinetic modeling or image registration. As such, no assumptions about tissue structure or function, or heterogeneities contained therein, are required for concentration analyses. This ability to longitudinally quantify blood pool CA concentration is unique to QUTE-CE MRI.

Transitioning QUTE-CE MRI for clinical use will require overcoming several additional challenges. Utilization on clinical machines may result in larger B_0 and B_1 inhomogeneity.

The placement of the coil can vary between patients on a clinical machine, which can lead to additional error resulting from the varying B_0 profile. Standardization of patient placement may be effective in reducing this error. B_1 inhomogeneity may be greater in larger coils, thus the extent to which B_1 inhomogeneity is present should be determined for x, y and z locations within the coil. We have shown that measurements taken at the coil center in vitro minimized this effect. A future improvement of the technique could include rapid B_1^+ mapping to correct for inhomogeneity (35) or use of a homogeneous phantom to accompany patients for back-calculating the flip angle along the z-axis.

These challenges may be offset by increases in sensitivity and a decreased scan time. The ferumoxytol operating range and precision should decrease as a function of magnetic field strength. For example, transitioning from a research grade (7T) to clinical grade (1.5T or 3T) scanner would lower the measurable concentration range by a couple of orders of magnitude due to increased CA r_1 relaxivity at lower field strengths (36), although organs may no longer be as invisible to UTE pulse sequences compared with 7T. The use of clinically available multichannel coils is expected to increase SNR and accelerate scan time.

Ferumoxytol-based blood-pool functional diagnostics may be the most immediate application of QUTE-CE MRI. Current functional MRI techniques, such as dynamic contrast-enhanced MRI, are unable to produce robust, reproducible results due to their inability to measure the intravascular CA concentration as a function of time (37,38). A 3D QUTE-CE image could be employed after a sequence of short two-dimensional (2D) images to provide a time point to better fit the model for the arterial input function. Alternatively, 2D QUTE-CE MRI could be used for more rapid scanning, provided sufficiently rapid gradient ramp-up time. However, imaging with a 2D sequence or 3D slice-select sequence would not benefit from the reduction in flow effects, which proved important for quantitative imaging by providing adequate homogeneity in flip angle excitation (i.e., whole sample excitation at low TR). Thus, we would recommend using a saturation prepulse before application of the slab or slice select pulse. Nevertheless, the effectiveness of 2D quantitative imaging with QUTE-CE is yet to be determined.

Quantification of CA accumulation in tissues is also of great clinical interest (39). It would be interesting to explore whether QUTE-CE MRI is compatible with other CAs, including various blood pool agents, organ-specific agents, and tumor-specific agents (28,40). CAs that produce significant T_1 shortening are likely to provide even higher contrast and precision than ferumoxytol. However, obtaining measurements outside of vasculature introduces additional complexities due to differences in CA relaxivity between blood, extracellular space, and intracellular compartments (41) and is the subject of future studies. It may also require an accurate R_{10} map before contrast administration. However, because any concentration measurement with MRI would have to overcome these additional complexities in tissue, we expect signal measurements from this method to maintain its advantages concerning the time-dependent process of signal perturbation that occurs at longer TEs. Wherever QUTECE MRI may prove successful, autosegmentation under user-specified conditions would provide unbiased, efficient delineation of features of interest (42).

CONCLUSIONS

The method described here allows clinically relevant concentrations of ferumoxytol to be measured noninvasively and quantitatively with high precision. We demonstrate that QUTE-CE MRI data show excellent agreement with theory when image acquisition parameters are preoptimized to reduce error. The robustness of this technique is based on the use of UTE, which allows the SPGR equation to be applied. Longitudinal measurements of blood pool ferumoxytol can be acquired in vivo with high precision for estimation of ferumoxytol half-life. This ability to longitudinally quantify blood pool CA concentration is unique to the QUTE-CE method and makes MRI competitive with nuclear imaging. We see immediate potential for diagnostic functional imaging with ferumoxytol. Future preclinical adaptations may include measurements of other CAs in organs outside the vasculature for image-guided drug delivery.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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FIG. 1.
Flow chart for ferumoxytol blood concentration measurements.

FIG. 2. 3D radial UTE images of ferumoxytol injection in mice. **a, b:** Precontrast (a) and postcontrast (b) image of the whole upper body (5 cm^3 isotropic FOV, $250\text{ }\mu\text{m}^3$ isotropic resolution, $\text{TE} = 13\text{ }\mu\text{s}$, $\text{TR} = 8\text{ ms}$, $\theta = 16^\circ$). **c, d:** Precontrast (c) and postcontrast (d) images of the thoracic region (3 cm^3 isotropic FOV, $150\text{ }\mu\text{m}^3$ isotropic resolution, $\text{TE} = 13\text{ }\mu\text{s}$, $\text{TR} = 3.5\text{ }\mu\text{s}$, $\theta = 20^\circ$).

FIG. 3.

Optimization of QUTE-CE image acquisition parameters. **a**: Heatmap of the standard error in concentration as a function of θ , TE, and TR. The lowest error is observed at $\theta = 20^\circ$, TE = 13 μs , and TR 3.5 ms. **b**: Variation in the measurement residual by changing TR, with the optimal curve shown in blue. Fixed parameters: $\theta = 20^\circ$ and TE = 13 μs . **c**: Variation in the measurement residual by changing TE, with the optimal curve shown in blue. Fixed parameters: $\theta = 20^\circ$ and TR = 3.5 ms. **d**: Agreement between measured signal intensity (circles) and theory (dashed line) under optimal image acquisition parameters for samples with known concentrations. **e**: Measured SNR (black) and CNR (gray) ratio as a function of ferumoxytol concentration under optimal image acquisition parameters.

FIG. 4.

Measurement of ferumoxytol concentration in whole calf blood phantoms. **a:** Measured $K\rho$ values (circles) and the calibration value (dotted line) set to the average value from doped vials demonstrates that $K\rho$ is constant for the concentration range of interest at optimal imaging parameters ($\theta = 20^\circ$, $\text{TE} = 13 \mu\text{s}$, $\text{TR} = 4 \text{ ms}$). **b:** Agreement between measured and actual ferumoxytol concentration for phantoms containing concentrations of ferumoxytol (circles). Line $y = x$ (dotted line) is shown for comparison. Inset: Linear regression residuals about $y = x$ for experimental measurements. **c:** 2D positive-contrast slice image from a 3D

optimized UTE pulse sequence. Phantoms contain 128, 96, 64, and 0 $\mu\text{g/mL}$ ferumoxytol, respectively (counterclockwise). **d:** Corresponding ferumoxytol concentration as calculated by theory. **e:** Concentration profile along the z-axis of the doped phantoms demonstrates the effect of B_1 inhomogeneity on concentration measurements. Measurements are always most precise in the center (z-axis slice position = 0).

FIG. 5.

Measurement of ferumoxytol concentration in vivo. **a:** Representative precontrast QUTE-CE image rendered with 3DSlicer, demonstrating that the mouse interior is invisible. **b:** Corresponding postcontrast image of a mouse treated with a 0.4- to 0.8-mg bolus of ferumoxytol, showing clear delineation of the thoracic vasculature. **c:** Automated segmentation, centered at one standard deviation of the measured mean, allows reconstruction of regions containing the contrast agent. **d:** Agreement between ferumoxytol concentration measured by QUTE-CE MRI (in vivo) and ICP-AES (ex vivo, of drawn blood). Inset: Measured residuals show excellent agreement, with an average of 7.07% error ($6.01 \pm 4.93 \mu\text{g/mL}$, maximum $13.5 \mu\text{g/mL}$ error). Inset: Representative 2D slice positive-contrast image demonstrating ROI placement for the QUTE-CE image analysis. **e:** Measured ferumoxytol blood concentration as a function of time, demonstrating sufficient accuracy to permit calculation of contrast agent half-life by imaging alone.

Table 1**Reported Precision of CA Quantification Using MRI**

Method	Percent error	CA	ROI	Scan time	Study
QUTE-CE In vivo	7.1% in vivo, 3.0% in vitro	Ferumoxylol (SPION)	Vasculature	8 min, 22s	Present study
Dynamic contrast-enhanced MRI with vascular input function	15.4% $\pm$ 4%	Gadolinium	Tissue	1.12 s/slice; 4 min, 40 s total	Walker-Samuel et al. (12)
Dynamic contrast-enhanced MRI	30.63%	Gadodiamide	Vasculature	~5 s/slice; 6–9 min total	Schabel and Parker (11)
Gradient echo fast field echo	12.7% $\pm$ 9.1%	SPION	Tumor	3.22 s/slice; 4 min, 18 s total	Langley et al. (31)
In vitro					
Delta relaxation enhanced MRI	8.2% $\pm$ 11.7%	Gadofluorine M	Phantom	12 min, 48 s/slice	Hoelscher et al. (32)
Susceptibility gradient mapping	11.14%	SPION	Phantom	2 min, 30 s	Zhao et al. (30)
Quantitative susceptibility imaging	1.2%	Gadolinium	Phantom	0.6 s/slice	de Rochefort et al. (9)
Rapid acquisition with refocuses echoes	24.13%	Fluorine based	Stem cells in pancreas	7 min, 46 s	Srinivas et al. (13)