

Low-Molecular-Weight Iron Chelates May Be an Alternative to Gadolinium-based Contrast Agents for T1-weighted Contrast-enhanced MR Imaging¹

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Purpose:

To synthesize two low-molecular-weight iron chelates and compare their T1 contrast effects with those of a commercial gadolinium-based contrast agent for their applicability in dynamic contrast material-enhanced (DCE) magnetic resonance (MR) imaging.

Materials and Methods:

The animal experiments were approved by the local ethics committee. Two previously described iron (Fe) chelates of pentetic acid (Fe-DTPA) and of trans-cyclohexane diamine tetraacetic acid (Fe-tCDTA) were synthesized with stability constants several orders of magnitude higher than those of gadolinium-based contrast agents. The T1 contrast effects of the two chelates were compared with those of gadopentetate dimeglumine in blood serum phantoms at 1.5 T, 3 T, and 7 T. For in vivo studies, a human breast cancer cell line (MDA-231) was implanted in five mice per group. The dynamic contrast effects of the chelates were compared by performing DCE MR imaging with intravenous application of Fe-DTPA or Fe-tCDTA on day 1 and DCE MR imaging in the same tumors with gadopentetate dimeglumine on day 2. Quantitative DCE maps were generated with software and were compared by means of a one-tailed Pearson correlation test.

Results:

Relaxivities in serum (0.94 T at room temperature) of Fe-tCDTA ($r_1 = 2.2 \text{ mmol}^{-1} \cdot \text{sec}^{-1}$, $r_2 = 2.5 \text{ mmol}^{-1} \cdot \text{sec}^{-1}$) and Fe-DTPA ($r_1 = 0.9 \text{ mmol}^{-1} \cdot \text{sec}^{-1}$, $r_2 = 0.9 \text{ mmol}^{-1} \cdot \text{sec}^{-1}$) were approximately twofold and fivefold lower, respectively, compared with those of gadopentetate dimeglumine ($r_1 = 4.1 \text{ mmol}^{-1} \cdot \text{sec}^{-1}$, $r_2 = 4.8 \text{ mmol}^{-1} \cdot \text{sec}^{-1}$). Used at moderately higher concentrations, however, iron chelates generated similar contrast effects at T1-weighted MR imaging in vitro in serum, in vivo in blood, and for DCE MR imaging of breast cancer xenografts. The volume transfer constant values for Fe-DTPA and Fe-tCDTA in the same tumors correlated well with those observed for gadopentetate dimeglumine (Fe-tCDTA Pearson R , 0.99; $P = .0003$; Fe-DTPA Pearson R , 0.97; $P = .003$).

Conclusion:

Iron-based contrast agents are promising as alternatives for contrast enhancement at T1-weighted MR imaging and have the potential to contribute to the safety of MR imaging.

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Gadolinium-based contrast agents (GBCAs) have been shown to be essential for magnetic resonance (MR) imaging of many disease states and to improve detection and characterization of disease in virtually all body parts. GBCAs are considered safe and effective. Complication rates after injection of GBCA are low, especially with regard to allergic or pseudoallergic reactions. Unlike iodine-based contrast agents for radiographic imaging modalities such as computed tomography (CT), GBCAs do not interfere with thyroid metabolism or cause kidney damage (contrast material-induced nephropathy).

Nephrogenic systemic fibrosis recently was identified as a late complication of GBCAs in individuals with reduced renal clearance. Impaired renal excretion of GBCA can lead to dechelation, especially in linear or group 1 GBCAs (1). Moreover, it has been shown that repeated administration of GBCAs leads to accumulation of GBCA in tissue, especially in bones, but also in certain areas of the human brain (globus pallidus and dentate nucleus), as is evident in increased signal intensity or reduced T1 of these tissues on T1-weighted MR images subsequently obtained without contrast medium administration (2).

Advances in Knowledge

- The iron chelates of trans-cyclohexane diamine tetraacetic acid (Fe-tCDTA) and pentetic acid (Fe-DTPA) can be used as low-molecular-weight T1 contrast agents for dynamic contrast-enhanced MR imaging in a breast cancer mouse model.
- When given at moderately higher doses (twofold for Fe-tCDTA and fivefold for Fe-DTPA), the iron chelates yielded similar contrast effects, and the dynamic contrast-enhanced volume transfer constants correlated well with that of gadopentetate dimeglumine (volume transfer constant for Fe-tCDTA; Pearson R , 0.99; P = .0003; volume transfer constant for Fe-DTPA R , 0.97; P = .003).

An increased risk of accumulation of GBCA in tissue with repeated GBCA administration was also shown in healthy rats (3). A more sensitive and quantitative mass spectrometric study revealed gadolinium deposits in the brain not only for these but even for more stable (non-group 1, macrocyclic) GBCAs in patients with normal renal function (4).

Although so far there is no evidence that the accumulation of traces of gadolinium is associated with clinical symptoms, the toxicity profile of free gadolinium suggests that deposition in human tissue, especially in brain tissue, should be avoided. Thus, despite all advances accomplished with GBCA-enhanced MR imaging, there is concern about the long-term safety of these agents. Accordingly, there is a potential clinical need for alternative contrast agents that can replace gadolinium-based chelates as T1-shortening contrast agents. These alternative agents should have the same T1 effects, while being non-toxic or far less toxic than free gadolinium, even when they are dechelated and accumulate locally.

Therefore, we investigated whether chelates loaded with paramagnetic ferric iron (Fe^{3+}) instead of paramagnetic gadolinium (Gd^{3+}) could serve as replacements for contrast material-enhanced T1-weighted imaging applications such as dynamic contrast-enhanced (DCE) imaging used, for example, in breast MR imaging. Although the short-term toxicity of elementary Fe^{3+} (5) is comparable to that of Gd^{3+} (6), the long-term toxicity of potentially released traces of Fe^{3+} should be lower, since the body contains approximately 4 g of iron (7) and has highly regulated systems for its safe transport and storage.

The first attempts to use iron-based chelates for contrast-enhanced MR imaging were made as early as 1985 (5,8,9), but they have not received much attention since. Because of the current concerns about GBCAs, we synthesized iron chelates of pentetic acid (Fe-DTPA) and of trans-cyclohexane diamine tetraacetic acid (Fe-tCDTA) meglumine according to a patent published by Gries et al (10). The chemical properties of Fe-DTPA and Fe-tCDTA are comparable to

those of gadopentetate dimeglumine (Gd-DTPA) and other GBCAs. The molecular models of Gd-DTPA, Fe-DTPA, and Fe-tCDTA allow visualization of similar sizes and structures of the chelates (Fig 1). They differ no more than the clinically established group of GBCAs themselves (11), which are used interchangeably for clinical MR imaging. Therefore, we anticipated that low-molecular-weight iron chelates could be used clinically with the same MR imaging protocols as are used for GBCA-enhanced MR imaging. Thus, the purpose of this study was to synthesize two low-molecular-weight iron chelates and compare their T1 contrast effects with those of a commercial GBCA for their applicability in DCE MR imaging. For that we chose Fe-tCDTA meglumine because of its relatively high r_1 relaxivity and Fe-DTPA because of its similarity to the well-established gadolinium-based Gd-DTPA.

Materials and Methods

Information on synthesis and characterization of the GBCAs and cell culture appears in Appendix E1 (online).

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Abbreviations:

DCE = dynamic contrast enhanced
 Fe-DTPA = iron chelate of pentetic acid
 Fe-tCDTA = iron chelate of trans-cyclohexane diamine tetraacetic acid
 GBCA = gadolinium-based contrast agent
 Gd-DTPA = gadopentetate dimeglumine
 K^{trans} = volume transfer constant

Author contributions:

Guarantors of integrity of entire study, P.B.S., R.H., E.A.S.; study concepts/study design or data acquisition or data analysis/interpretation, all authors; manuscript drafting or manuscript revision for important intellectual content, all authors; approval of final version of submitted manuscript, all authors; agrees to ensure any questions related to the work are appropriately resolved, all authors; literature research, P.B.S., A.H., R.H., E.A.S.; clinical studies, R.H.; experimental studies, P.B.S., A.H., R.H., S.M., E.A.S.; statistical analysis, P.B.S., R.H., E.A.S.; and manuscript editing, P.B.S., A.H., R.H., C.K.K., E.A.S.

Conflicts of interest are listed at the end of this article.

See also Science to Practice by Tweedle in this issue.

Figure 1

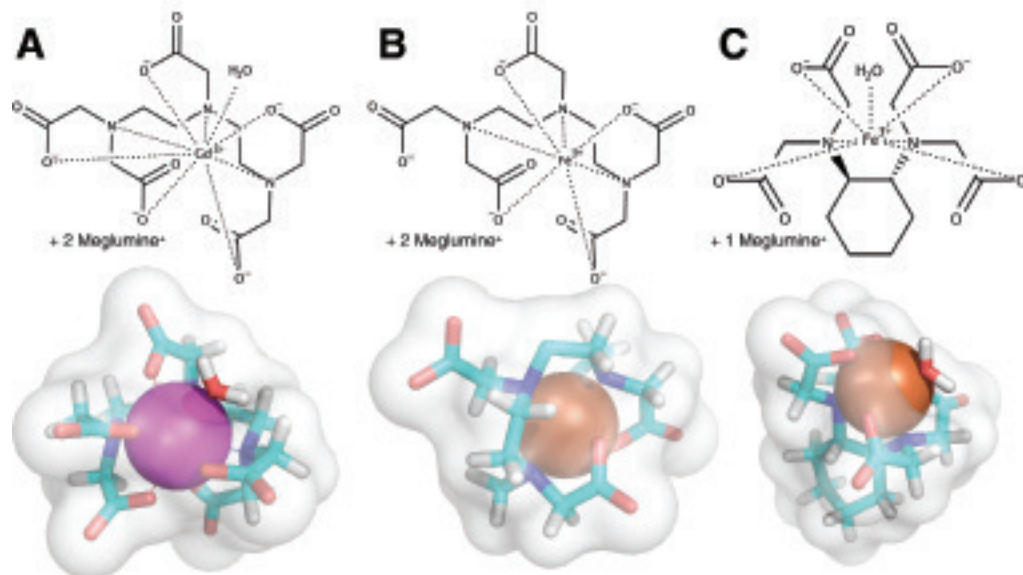


Figure 1: Illustrations show molecular structures and three-dimensional models of, *A*, the investigated Gd-DTPA, *B*, Fe-DTPA, and, *C*, Fe-tCDTA. Structures show nine coordination sites of Gd³⁺ and seven of Fe³⁺. *A*, Gd-DTPA (11) and, *C*, Fe-tCDTA (12) leave one side available for water coordination, which explains relatively high relaxivity. *B*, Fe-DTPA (13) has lower relaxivity; all seven coordination sites of Fe³⁺ are occupied. Magenta = gadolinium, brown = iron, cyan = carbon, red and orange = oxygen, blue = nitrogen, gray = hydrogen.

MR Imaging of Contrast Agent Phantoms

The T1 contrast effects of Fe-DTPA, Fe-tCDTA, and Gd-DTPA (Magnevist; Bayer, Leverkusen, Germany) were compared in MR imaging phantom experiments with fetal calf serum as a blood-like environment at room temperature. To reveal a potential influence of the field strength, we imaged the samples with 1.5-T, 3-T, and 7-T MR imaging units. The phantoms were prepared by arranging six MR spectroscopic tubes containing fetal calf serum in a circular position. Starting with the upper tube, the samples contained clockwise increasing concentrations of the chelates (in millimoles of Fe³⁺ or Gd³⁺ per liter): 0.03, 0.1, 0.3, 1.0, 3.0, and 10.0 mmol/L. A tube in the center contained only fetal calf serum as the control. Imaging parameters are provided in Table 1.

Mouse Tumor Xenograft Model and Study Design

Athymic nude mice (5–6-weeks old) were obtained from Charles River Laboratories (Sulzfeld, Germany) and were

housed according to the guidelines of the Animal Welfare Committee of the Charité-Universitätsmedizin Berlin. All experiments were approved by the Regional Office for Health and Social Affairs Berlin (LAGeSo, license number G 0285/13).

Human breast cancer cells (MDA-231) were injected subcutaneously into the flanks, and tumor growth was observed for 3–4 weeks, until the tumor size reached at least 5 mm. Seven animals underwent DCE MR imaging in a single session for proof-of-concept imaging at 1.5 T. For comparison of agents at 7 T, animals underwent DCE MR imaging on 2 consecutive days, and all animals for which tail vein injections were successful on day 1 (Fe-DTPA or Fe-tCDTA) and day 2 (Gd-DTPA) were included in the analysis (two groups of five mice).

In Vivo MR Imaging at 1.5 T

Proof-of-concept imaging was performed with a clinical 1.5-T MR imaging device (Sonata; Siemens, Erlangen, Germany). The contrast agents were administered intravenously through a tail vein

catheter at a dose of 0.2 mmol/kg or 0.5 mmol/kg for Fe-tCDTA, a dose of 0.5 mmol/kg for Fe-DTPA, and a dose of 0.1 mmol/kg for Gd-DTPA. By using a modified clinical DCE MR imaging sequence, the contrast agents were injected during a delay of 30 seconds after a preimaging sequence of 61 seconds through a tail vein catheter, followed by 12 repetitions (12 minutes) of a fast low-angle shot sequence (Table 1). The T1 contrast effects of the chelates were compared by evaluating the signal intensity regions of interest in the left heart chamber before and after chelate injection at the signal intensity peak.

In Vivo DCE MR Imaging at 7 T

All imaging parameters are provided in Table 1. For in vivo MR imaging at 7 T, tumor-bearing mice were anesthetized (Appendix E1 [online]) and imaged by using a dedicated small-animal MR imaging unit (BioSpec; Bruker, Ettlingen, Germany). First, the tumor was located during a T2-weighted sequence. DCE MR images were acquired with a fast low-angle shot sequence in three contiguous

Table 1

MR Imaging Acquisition Parameters

Parameter	Phantom, 1.5 T and 3 T	DCE, 1.5 T	Anatomy, 1.5 T	Phantom, 7 T	DCE, 7 T	VFA T1 Map, 7 T	Anatomy, 7 T
Pulse sequence	FLASH 3D	FLASH 3D	FLASH 2D	FLASH 2D	FLASH 2D	FLASH 2D	RARE 2D (RARE factor 8)
Tx/Rx radiofrequency coil setup	Solenoid (Tx/Rx)	Solenoid (Tx/Rx)	Solenoid (Tx/Rx)	Ø 40 mm diameter birdcage (Tx/Rx)	Ø 86 mm birdcage (Tx), mouse head surface coil (Rx)	Ø 86 mm birdcage (Tx), mouse head surface coil (Rx)	Ø 86 mm birdcage (Tx), mouse head surface coil (Rx)
Field of view (mm)	200 × 200 × 128	100 × 50 × 64	100 × 100	25.6 × 25.6	30 × 30	30 × 30	30 × 30
Image matrix	256 × 128 × 64	256 × 128 × 64	256 × 128	128 × 128*	128 × 128*	128 × 128*	256 × 256
No. of sections	NA	NA	18	3	3	3	32
Section thickness (mm)	NA	NA	1.5	2	2	2	1
Repetition time (msec)	8.5	8.5	200	25	25	25	3500
Echo time (msec)	2.7	4.5	5.4	2	2	2	33
Flip angle (degrees)	17	25	15	30	30	5, 15, 30, 40, 60	90/180
Averages	1	1	8	8	8	8	4
No. of repetitions	1	12	10	10	330	1	1
Acquisition time	1 min 10 sec	12 min 44 sec	3 min 24 sec	2.15†	11 min 49 sec	10.75†	7 min 28 sec

Note.—Ø = diameter, 2D = two-dimensional, 3D = three-dimensional, FLASH = fast low-angle shot, NA = not available, RARE = rapid acquisition with relaxation enhancement, Rx = receive, Tx = transmit, VFA = variable flip angle.

* Acquisition matrix 89 × 69 interpolated to 128 × 128 by partial Fourier and zero filling.

† Data are presented in seconds.

sections through the tumor (Table 1). For T1 mapping, a set of images with variable flip angles was acquired, followed by the DCE sequence. The contrast agent was injected through a tail vein catheter after acquisition of 30 baseline images (Fe-tCDTA and Fe-DTPA at 0.5 mmol/kg of body weight). For comparison of iron complexes with Gd-DTPA in the same tumor, DCE MR imaging was performed 24 hours later by using Gd-DTPA at 0.1 mmol/kg of body weight (12).

Analysis of DCE MR Imaging and Comparison of Iron Chelates with Gd-DTPA

Quantitative DCE maps were generated with software (Matlab 2014a; MathWorks, Natick, Mass) and the Rocketship Toolbox (13) by using the Patlak model, which recently was shown to be less error prone (14). Because r_1 relaxivities do not change excessively throughout field strengths (eg, for Gd-DTPA they range from 3.8 to 4.1 L · mmol⁻¹ · sec⁻¹ for field strengths from 0.47 T to 1.5 T) (15), these were chosen from serum values at 0.94 T (Table 2). The arterial input function was measured in the abdominal aorta. For qualitative

Table 2

Relaxivity of Gd-DTPA, Fe-DTPA, and Fe-tCDTA at 0.94 T

Contrast Agent	r_1 (L · mmol ⁻¹ · sec ⁻¹)		r_2 (L · mmol ⁻¹ · sec ⁻¹)	
	In Water	In Serum	In Water	In Serum
[Meglumine] ₂ Gd-DTPA	3.4	4.1	3.9	4.8
[Meglumine] ₂ Fe-DTPA	0.6	0.9	0.6	0.9
[Meglumine] Fe-tCDTA	2.0	2.2	2.2	2.5

comparison, relative volume transfer constant (K^{trans}) values were calculated voxelwise and maps were color coded, interpolated, and overlaid on T2-weighted images. For quantitative analysis, K^{trans} and contrast-to-noise ratio (post- vs pre-injection images) were measured in a region of interest drawn around the tumor core in the central section, excluding the heterogeneous tumor rim.

Statistical Analysis

For each animal, mean K^{trans} and contrast-to-noise ratios of the iron complex were plotted against those measured with Gd-DTPA and were linearly fitted (no intercept) and slopes ± confidence intervals and corresponding P values

were calculated by using software (Prism; GraphPad Software, La Jolla, Calif) followed by a one-tailed Pearson correlation test.

Results

Properties of the Iron Chelates

The relaxivity values of Fe-DTPA, Fe-tCDTA, and Gd-DTPA are summarized in Table 2. In accordance with previously published data (8), the relaxivities of the iron chelates were lower than those of Gd-DTPA. For both iron compounds and for Gd-DTPA, the relaxivity in bovine serum was higher than that in water, which likely was caused by

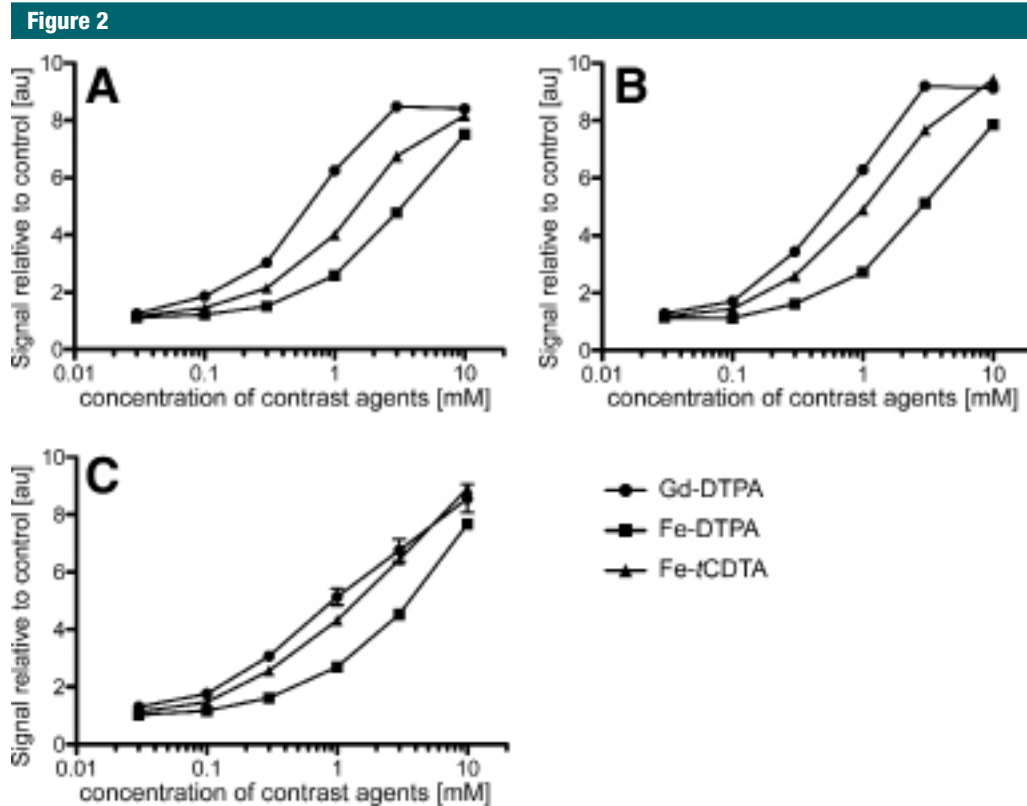


Figure 2: Graphs show concentration dependency of T1 contrast effects of Gd-DTPA, Fe-DTPA, and Fe-tCDTA at different field strengths. Signal intensity ratios of T1-weighted MR images of serum phantoms with increasing concentrations of contrast agents were acquired at, A, 1.5 T, B, 3 T, and, C, 7 T by using a typical DCE pulse sequence at room temperature. Images of the serum phantoms are shown in Figure E1 (online).

different viscosity of the serum (15). The iron chelate preparations were stable without precipitation (eg, iron hydroxide) for at least 15 months at room temperature, concentrations of one-half mole per liter and neutral pH.

The structures and molecular models of the three chelates are shown in Figure 1. The double-negative charge of Gd-DTPA and Fe-DTPA and the single-negative charge of Fe-tCDTA is countered by meglumine. As for those in Gd-DTPA but contrary to those in Fe-DTPA, where all metal coordination sides are occupied by the chelator, one coordination side of Fe-tCDTA is available for exchangeable coordination of water molecules (Fig 1), contributing to the relatively high T1 relaxivity (r1).

MR Imaging of Contrast Agent Phantoms

As expected, when the same concentrations were used, the signal intensity

increase on the T1-weighted images was lower for the iron chelates than for Gd-DTPA (Fig 2). Figure E1 (online) shows images of the phantoms. However, with higher contrast agent concentrations, the T1 effect was comparable. The required concentration increases to achieve a similar signal intensity enhancement as that with Gd-DTPA were approximately twofold for Fe-tCDTA and approximately fivefold for Fe-DTPA at 1.5 T, which is in the range expected on the basis of the relaxivity measurements at 0.94 T (Table 2). The contrast effect differences of the iron chelates, especially that of Fe-tCDTA, slightly diminished at higher field strengths.

In Vivo DCE MR Imaging of Mouse Tumor Xenografts

Figure 3 shows examples of two mice that were examined with either Fe-DTPA or Fe-tCDTA on the 1st day and with

Gd-DTPA for comparison on the 2nd day. The T2-weighted MR imaging sequence was followed by T1-weighted imaging before and after contrast agent injection for generation of subtraction images of the implanted tumors 3–4 weeks after tumor induction (Fig 3, A–D). The postcontrast images with the highest mean signal intensity in the tumor are shown. Signal intensity and concentration time curves in tumors and arteries (Fig E3 [online]) allowed computation of DCE parametric maps. An important quantitative DCE parameter is K^{trans} , which is a measure of the rate at which a contrast agent crosses from plasma into the tissue compartment per minute and is sensitive to a combination of parameters, especially for vascular flow and permeability. Semiquantitative comparison of iron complex K^{trans} maps with Gd-DTPA maps in the same animals showed a similar heterogeneous pattern with increased K^{trans} in the tumor

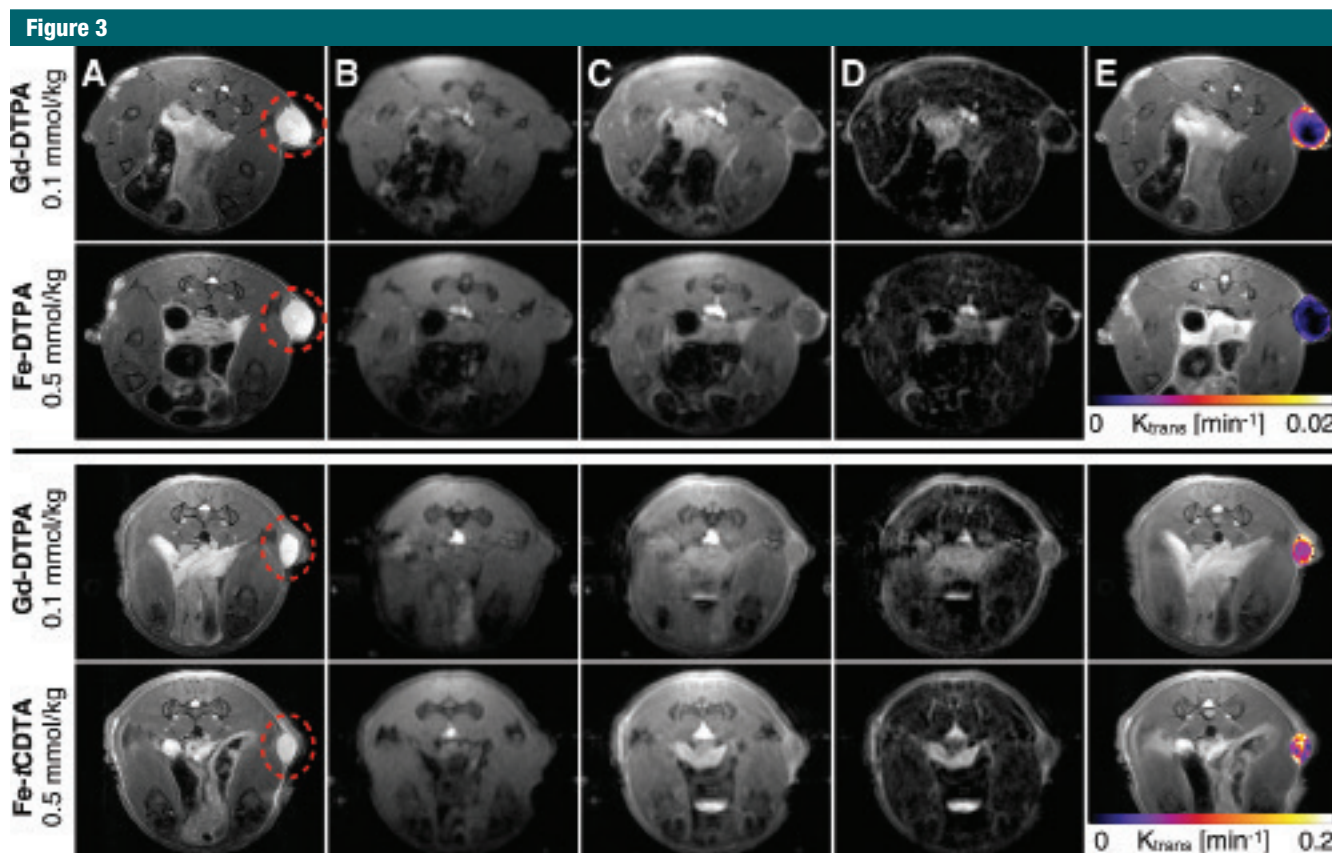


Figure 3: MR images show in vivo T1 contrast effects and DCE MR imaging parameter mapping with Fe-DTPA and Fe-tCDTA compared with those of Gd-DTPA at 7 T. Images represent two of 10 mice examined with Fe-DTPA on the 1st day and with Gd-DTPA on the next day (upper two rows, $n = 5$) or with Fe-tCDTA on the 1st day and Gd-DTPA on the next day (lower two rows, $n = 5$): A, T2-weighted preinjection images with tumors indicated by dashed circles; B, T1-weighted preinjection images in same position; C, T1-weighted images several minutes after injection in same position; D, Subtraction images of B and C; and E, color-coded K^{trans} parametric maps of tumor overlaid on T2-weighted images. Images of eight other mice are shown in Figure E2 (online).

rim and lower K^{trans} in the tumor core, which indicated higher permeability, vascular flow, or a combination in the tumor rim (Fig 3, E).

Because of the heterogeneity close to the rim, which complicates comparison and selection of regions of interest on the 2 consecutive days, we quantitatively compared mean K^{trans} and contrast-to-noise ratios in the tumor core region by using a linear model with no intercept (Fig 4). The following relations were found: Fe-tCDTA $K^{\text{trans}} = (1.17 \pm 0.06) \cdot \text{Gd-DTPA } K^{\text{trans}}$ ($P = .0002$) and Fe-DTPA $K^{\text{trans}} = (0.59 \pm 0.05) \cdot \text{Gd-DTPA } K^{\text{trans}}$ ($P < .0002$); Fe-tCDTA CNR = $(1.25 \pm 0.15) \cdot \text{Gd-DTPA CNR}$ ($P = .001$) and Fe-DTPA CNR = $(0.58 \pm 0.03) \cdot \text{Gd-DTPA CNR}$ ($P < .0001$), where CNR is the contrast-to-noise ratio. Iron

complex K^{trans} values were well correlated with those of the Gd-DTPA experiments in the same mouse 24 hours later (Fe-tCDTA Pearson R , 0.99; $P = .0003$; Fe-DTPA Pearson R , 0.97; $P = .003$). For the contrast-to-noise ratio, a significant correlation was only observed for Fe-DTPA (R , 0.98; $P = .001$) because of the low dynamic range of values in the Fe-tCDTA group (R , 0.25; $P = .34$).

To confirm in vivo that the T1-shortening effects of Fe-DTPA and Fe-tCDTA are also observable with a clinical 1.5-T system, we performed DCE MR imaging experiments comparable to the acquisitions above, but with a temporal resolution of 61 seconds per dynamic frame, which corresponds to the temporal resolution of currently used breast MR imaging protocols (Fig 5).

Again, by using higher concentrations of iron complexes (at 0.5 mmol/kg), the tumors showed a signal intensity increase with use of Fe-DTPA and Fe-tCDTA that was comparable to that achieved with Gd-DTPA (at 0.1 mmol/kg). Even a dose of 0.2 mmol/kg of Fe-tCDTA caused clear signal intensity increases on T1-weighted images compared with signal intensity on precontrast images. As with Gd-DTPA, the iron chelates were excreted into the bladder 10 minutes after injection.

The T1 contrast effect in blood is illustrated in Figure 6, which shows the hearts of the mice before and after intravenous injection of the low-molecular-weight chelates. While the imaging sequences were not optimized for MR angiography, the heart chambers were easily identified and demonstrated

Figure 4

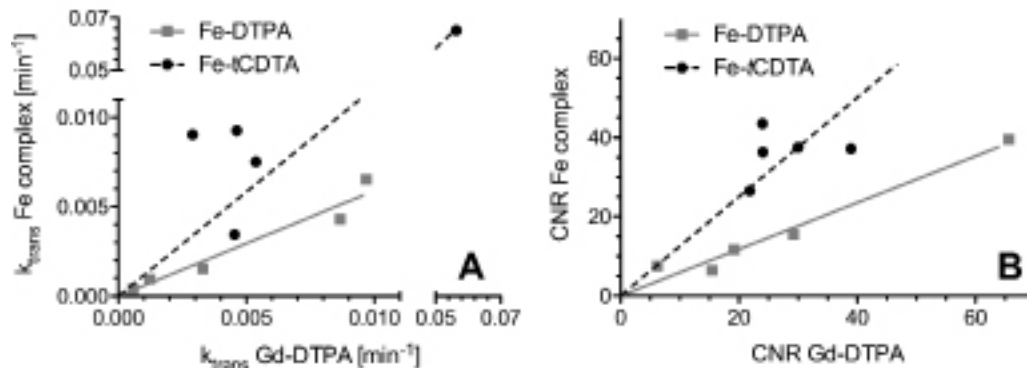


Figure 4: Graphs show correlation of k^{trans} values and contrast-to-noise ratios (CNRs) determined by using iron complexes and Gd-DTPA. *A*, Tumor core k^{trans} values and, *B*, contrast-to-noise ratios for each of the iron complexes plotted over Gd-DTPA and linear fit with no intercept.

Figure 5

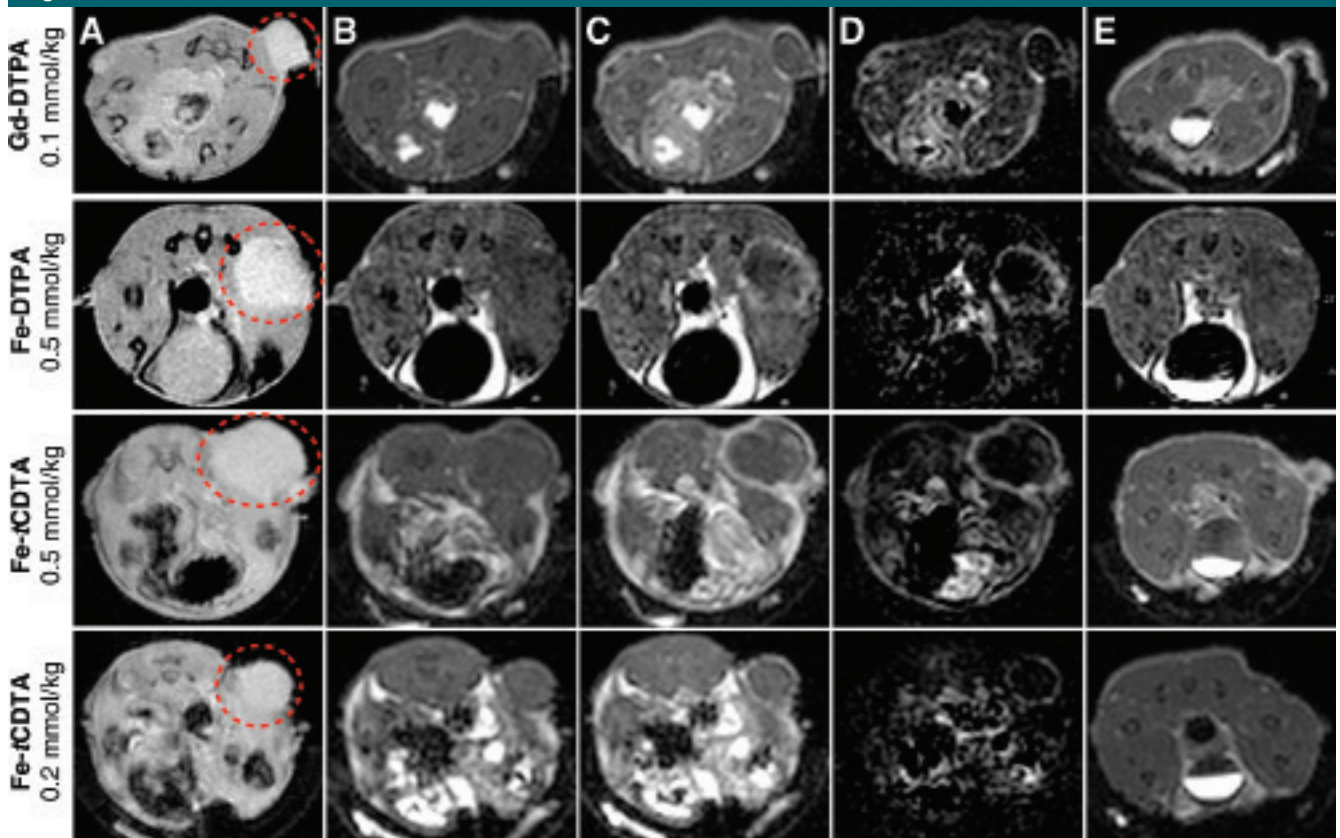


Figure 5: Images show examples of in vivo T1 contrast effects of Gd-DTPA, Fe-DTPA, and Fe-ICDTA at 1.5 T: *A*, T2-weighted preinjection images with tumors indicated by dashed circles; *B*, T1-weighted preinjection images in same position; *C*, T1-weighted images acquired approximately 3 minutes after injection in the same position; *D*, subtraction images of *B* and *C*; and, *E*, bladder approximately 10 minutes after injection.

signal intensity increases as expected from the relaxivity measurements and phantom experiments. Although only

two mice per group were imaged at 1.5 T, these preliminary data suggest that the iron chelates produce a blood signal

intensity increase in the left ventricles that is equal to that of Gd-DTPA. The mean $\pm$ standard deviation pre- and

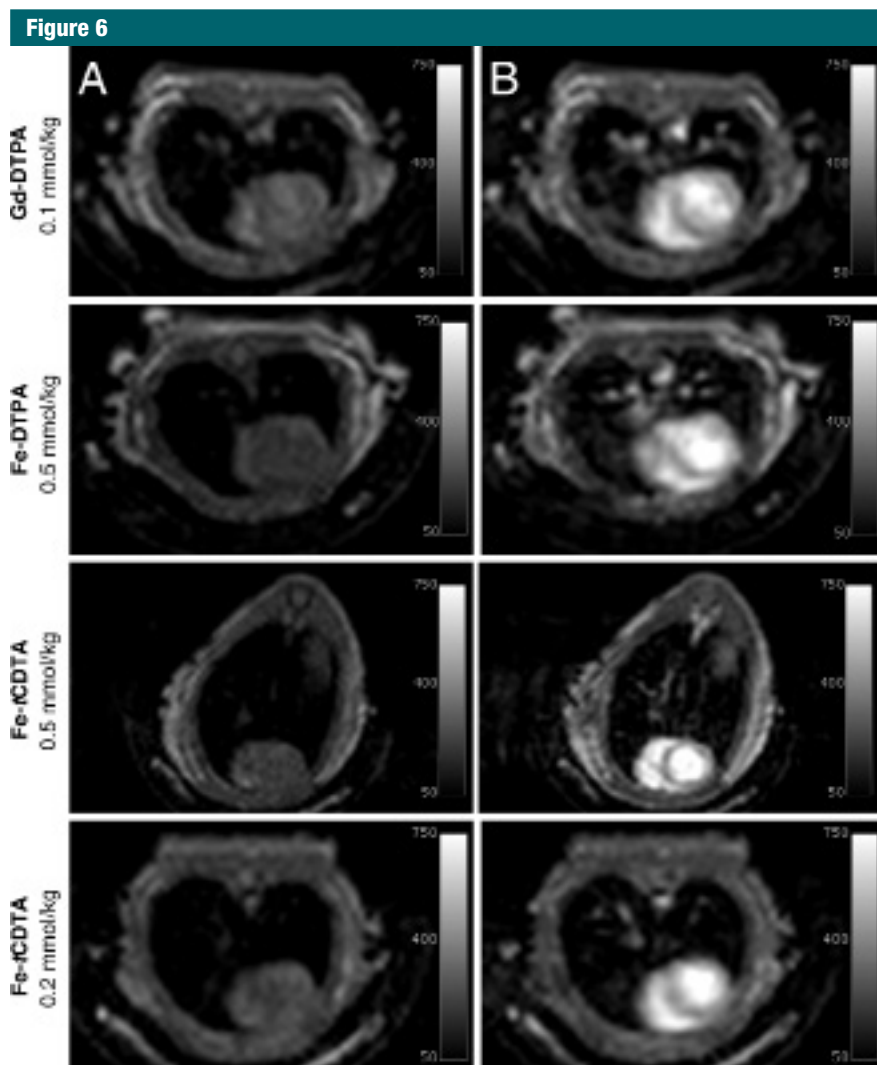


Figure 6: MR images of mouse hearts show T1 contrast effect of Gd-DTPA, Fe-DTPA, and Fe-tCDTA at 1.5 T. *A*, Images of mouse hearts before and, *B*, after injection of contrast agents show feasibility of T1-weighted MR angiography with iron chelates.

postcontrast agent signal-to-noise ratios were 1.97 ± 0.21 for Gd-DTPA (0.1 mmol/kg), 2.90 ± 0.82 for Fe-DTPA (0.5 mmol/kg), 3.30 for Fe-tCDTA (0.5 mmol/kg, one mouse only), and 2.75 ± 0.04 for Fe-tCDTA (0.2 mmol/kg). After the intravenous bolus injections, no adverse reactions were observed in the mice during examinations or after they awoke from anesthesia.

Discussion

In our study, we provided initial evidence that low-molecular-weight iron chelates

have the potential to be used for T1-weighted MR imaging and quantitative DCE MR imaging as alternatives to GBCAs. Results of our ex vivo phantom experiments and in vivo small-animal imaging studies in a mouse model of breast cancer suggest that the T1-shortening effects of Fe-DTPA and Fe-tCDTA at the doses we used in contrast-enhanced MR imaging studies were similar to those of Gd-DTPA, the standard of reference. We reproduced previously published relaxivity values of Fe-DTPA and Fe-tCDTA in water (8) and also measured relaxivity values in

serum, which resembles the blood environment more closely. The relaxivities of the iron chelates were in the same order of magnitude as those of Gd-DTPA. Consequently, in T1-weighted MR imaging measurements of concentration series in serum, the iron chelates produced similar MR imaging signals as did Gd-DTPA when concentrations were increased twofold for Fe-tCDTA and fivefold for Fe-DTPA. The contrast disadvantage of iron chelates seemed to be reduced at higher field strengths, which is attributable to the lower T2 relaxivity values of the iron chelates compared with gadolinium, for which an increase of r_2 with field strength is known (16). One could speculate that the lower MR imaging field strengths available in past studies (eg, 0.35 T [8]) and poor image quality discouraged further investigation of iron chelates for MR imaging.

We proceeded to investigate the utility of the iron chelates for DCE MR imaging in vivo. To account for tumor heterogeneity, we compared the T1 contrast effects of the iron chelates with those of Gd-DTPA in the same tumors at subsequent examinations on 2 successive days to ensure complete clearance of the chelates from the blood (12). Since vessel leakiness may change with tumor growth, tumors were always first imaged with iron complexes, then, 24 hours later, with Gd-DTPA. With the dose adjustments we have described, the T1 contrast effect of iron chelates (at 0.5 mmol/kg) was comparable to that observed for Gd-DTPA (at 0.1 mmol/kg) and resulted in very similar K^{trans} as a measure of the leakiness of the tumor blood vessels. The subtle differences in the K^{trans} might have been due to their slightly different molecular structures and charges. However, the contrast enhancement kinetics of the iron and gadolinium chelates were similar enough to use the same pulse sequence parameters for T1-weighted imaging: an important prerequisite for clinical acceptance. Even when used with a clinical 1.5-T system, iron chelates generated sufficient T1 contrast in vivo, as the proof-of-concept data on a clinical system show. A strong excretion into the bladder was observed for all

three contrast agents 10 minutes after injection.

Although the acute toxicity of the iron chelates was reported to be comparable to that of gadolinium chelates (17) (which must be investigated in more detail in the future), the long-term toxicity of iron chelates associated with the potential release of iron traces from the chelates should be lower than that of free gadolinium because of the well-regulated iron metabolism. Moreover, the stability constants of Fe-DTPA ($\log K = 27.3$) and Fe-CDTA ($\log K = 27.5$) are several orders of magnitude higher than those of Gd-DTPA ($\log K = 22.5$) (9) and other GBCAs (18). In fact, the replacement of gadolinium with iron, which is called transmetalation, could be one mechanism of gadolinium release from the chelates in the body (19,20). Furthermore, the risk of an increased concentration of free iron seems low, since the main carrier of plasma iron (between 9 $\mu\text{mol/L}$ and 36 $\mu\text{mol/L}$ of Fe) transferrin with stability constants of $\log K_1 = 22.7$ and $\log K_2 = 22.1$ (21) potentially releases constantly comparable amounts of iron as Fe-CDTA and Fe-DTPA at the peak concentrations after intravenous injection (for 0.5 mmol/kg corresponds to approximately 10 mmol/L of iron in plasma) that have approximately 100 000-fold higher stability constants. Therefore, we believe that, for the long term, iron chelates could be a safer alternative to GBCAs, but the acute and long-term toxicity needs to be investigated extensively in future studies. In our study, we did not observe any adverse reactions after bolus injections of the iron chelates. Finally, use of iron chelates instead of gadolinium chelates could pave the way for the development of T1 contrast agents that are not rapidly excreted (eg, blood pool agents, protein binders, and target-specific molecular imaging probes). Because longer persistence in the body increases the likelihood of dechelation, iron should be a safer alternative for such contrast agents.

Our study had several limitations. We reported about the comparison of two iron chelates with a GBCA at DCE MR imaging in an animal model

of breast cancer that included some proof-of-concept images of the T1 blood contrast. However, short- and long-term cell and animal toxicity must be investigated in detail in future studies. Furthermore, the contrast effects remain to be confirmed in other animal models, species, and imaging protocols to make conclusions about future clinical applicability.

In conclusion, our results suggest that Fe-DTPA and Fe-CDTA, at moderately higher concentrations, can be used as low-molecular-weight T1 contrast agents (eg, for DCE MR imaging of tumors). The contrast effects and enhancement kinetics of iron chelates were similar to those observed after Gd-DTPA administration. If future studies can provide proof of their safety, iron chelates could allow one of the greatest concerns of GBCA, the possible long-term toxicity, to be eliminated.

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