MAJOR PAPER

Intraindividual Crossover Comparison of Gadoteric Acid Dose for Liver MRI in Normal Volunteers

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(Received January 15, 2015; Accepted March 25, 2015; published online June 23, 2015)

Purpose: We performed a quantitative intraindividual comparison of the performance of 0.025- and 0.05-mmol/kg doses for gadoteric acid-enhanced liver magnetic resonance (MR) imaging.

Materials and Methods: Eleven healthy volunteers underwent liver MR imaging twice, once with a 0.025- and once with a 0.05-mmol/kg dose of gadoteric acid. MR spectroscopy and 3-dimensional gradient-echo T₁-weighted images (3D-GRE) were obtained before and 3, 10, and 20 min after injection of the contrast medium to measure T₁ and T₂ values and signal-to-noise ratio (SNR) and contrast-to-noise ratio (CNR) performance. During the dynamic phase, highly time-resolved 3D-GRE was used to estimate the relative CNR (CNR_{rel}) of the hepatic artery and portal vein (PV) to the liver. We used paired t-tests to compare the results of different doses.

Results: During the hepatobiliary phase, we observed shorter T₁ values and higher SNRs of the liver ($P < 0.001$) and higher liver-to-PV and liver-to-muscle CNRs ($P < 0.002$) using 0.05 mmol/kg compared to 0.025 mmol/kg. Increasing the dose to 0.05 mmol/kg yielded a greater T₁-shortening effect at 10 min delay even compared with 0.025 mmol/kg at 20 min ($P < 0.001$). During the dynamic phase, the peak CNR_{rel} for the hepatic artery and portal vein were higher using 0.05 mmol/kg ($P = 0.007$ to 0.035).

Conclusion: Use of gadoteric acid at a dose of 0.05 mmol/kg leads to significantly higher SNR and CNR performance than with 0.025 mmol/kg. Quantitatively, a 10-min delay may be feasible for hepatobiliary-phase imaging when using 0.05 mmol/kg of gadoteric acid.

Keywords: *gadoteric acid, liver, magnetic resonance imaging, relaxometry, spectroscopy*

Introduction

Gadoteric acid is a gadolinium-based contrast agent used primarily for hepatobiliary imaging applications, and it is now widely accepted as a pri-

mary contrast agent in magnetic resonance (MR) imaging of the liver.^{1,2} Its pharmacokinetics is similar to that of extracellular contrast agents immediately after bolus injection; it is taken up rapidly into hepatocytes and subsequently excreted into bile. Recent studies have demonstrated important advantages of gadoteric acid-enhanced MR imaging over other methods for imaging liver disease.^{3–6}

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Typically, maximum liver-to-lesion contrast is obtained in the hepatobiliary phase, approximately 20 min after injection, and good liver-to-lesion contrast persists about 2 to 3 hours.⁷ In the hepatobiliary phase, normal liver and bile appear hyperintense from the presence of gadolinium, whereas blood vessels and focal liver lesions appear hypointense. This contrast enables detection of even very small hepatic metastases or hepatocellular carcinoma as hypointense lesions in bright liver tissue.^{2,8–12} The use of hepatobiliary-phase images for the evaluation of hepatic function has also been suggested.^{13,14}

The approved package insert dose of gadoxetic acid, 0.025 mmol/kg, yields a quarter of the dose of gadolinium of traditional formulation agents, including gadopentetate dimeglumine, gadoteridol, gadodiamide, gadoversetamide, and gadobenate dimeglumine.¹⁵ This dose was determined based on preclinical studies^{16,17} that demonstrated comparable early enhancement of hypervascular tumors between MRI with a 0.025 mmol/kg dose showed comparable early enhancement of hypervascular tumors to gadopentetate dimeglumine.¹⁶ A dose of 0.025 mmol/kg was also found to be the minimum effective dose during the hepatobiliary phase for the detection and characterization of lesions.¹⁷ However, the imaging sequences used in these studies (both published in 1996) are no longer routinely used in clinical MR imaging. In fact, many institutions have adopted the use of higher doses, such as a fixed dose of 10 mL rather than a dose based on patient weight, as a standard in clinical MR imaging.^{18–20} For patients weighing 50 to 100 kg, this results in effective doses of 0.025 to 0.05 mmol/kg. Considering that a dose of 0.025 mmol/kg of gadolinium is only a quarter of the conventional MR contrast agent dose, the expected risk of toxicity from dose escalation is likely to be minimal.

The growing use of doses that exceed the package insert dose motivated a systematic re-evaluation of higher dosing of gadoxetic acid when used with state-of-the-art MR imaging sequences. Greater arterial and tumor enhancement in the early dynamic phase is expected using a higher dose of 0.05 mmol/kg than the 0.025 mmol/kg, and this may improve the detection and characterization of small enhancing liver lesions. Moreover, a recent study demonstrated improved clinical performance of gadoxetic acid at a dose of 0.05 mmol/kg compared to 0.025 mmol/kg for both dynamic- and hepatobiliary-phase imaging in patients with cirrhosis.²¹

The purpose of this crossover study was to perform a systematic and objective comparison of

doses of 0.025 mmol/kg and 0.05 mmol/kg of gadoxetic acid in healthy volunteers. We compared the doses in both the dynamic and hepatobiliary phases using state-of-the-art pulse sequences and included measurements of signal-to-noise ratio (SNR) and contrast-to-noise ratio (CNR). Further, we used MR spectroscopy-based relaxometry to measure the fundamental relaxation parameters of the liver after the administration of the contrast medium to facilitate optimization of future acquisition strategies.

Materials and Methods

Subjects

This prospective crossover study complied with Health Insurance Portability and Accountability Act (HIPAA) requirements and was approved by our institutional review board (IRB). We recruited 11 healthy subjects (4 men, 7 women; aged 23 to 64 years, mean, 38 ± 15 years; weight 54 to 91 kg, mean 70 ± 13 kg) from a local IRB-approved database of healthy volunteers and obtained their written informed consent prior to scans. We screened all volunteers to confirm they had no liver or renal disease.

The SNR and CNR measurements performed for hepatobiliary-phase images at 20 min after injection of 0.025 mmol/kg of gadoxetic acid in this study (see below) have also been used in a separate unrelated study.²² However, the comparison between 2 doses of gadoxetic acid has not been previously investigated in these subjects.

Study protocol and contrast agent

This crossover study comprised 2 visits separated by more than 4 weeks (28 to 76 days, mean 57 days). At the first visit, gadoxetic acid-enhanced liver MR imaging was performed using 0.025 mmol/kg (0.1 mL/kg) of gadoxetic acid (Eovist®, Bayer Healthcare). At the second visit, the same imaging protocol was repeated using a dose of 0.05 mmol/kg (0.2 mL/kg). The 0.025 mmol/kg dose was diluted 1:1 with saline (0.9% NaCl) to ensure injection of the same volume as for the 0.05 mmol/kg dose (off-label use for the study). Injections were performed using a power injector (Spectris Solaris®, MedRad®, Inc., Warrendale, PA, USA) at 2 mL/s by means of a 20-gauge antecubital intravenous catheter followed by a 20-mL saline chaser at the same injection rate. The volume and injection rate for each protocol were the same to ensure accurate comparisons of the time to peak enhancement as well as enhancement during the dynamic phase.²³

MR scanner

All imaging was performed on a clinical 3-tesla MR system (MR750, v23, GE Healthcare, Waukesha, WI, USA) using a 32-channel phased-array abdominal coil (Neocoil, Pewaukee, WI, USA).

MR spectroscopy-based relaxometry

MRS was performed before and 3, 10, and 20 min after the injection of gadoxetic acid to measure liver T_1 and T_2 values. A single-voxel stimulated echo acquisition mode (STEAM) MRS sequence was acquired in a single 21-s breath-hold. This STEAM sequence used varying repetition times (TRs; 150 to 1000 ms) to enable calculation of the T_1 value of the object. Multiple echo times (TEs; 10 to 110 ms) were also acquired to measure T_2 values of the object. These multiple acquisitions (multi TR and multi TE) were completed within a single breath-hold to enable independent measurement of the T_1 and T_2 of water and fat (if present).^{24,25} The MRS voxel was $20 \times 20 \times 20 \text{ mm}^3$ and was placed in the right lobe (segments 6 or 7) of each subject, avoiding large blood vessels or bile ducts. Other acquisition parameters included: receiver bandwidth, $\pm 2500 \text{ Hz}$; number of acquired points, 256; total number of spectra acquired, 32 (including 4 prepulse excitations to reach steady state).

MR imaging

A 3-dimensional (3D) T_1 -weighted MR sequence was used before and 3, 10, and 20 min after contrast injection using parameters: breath-hold T_1 -weighted 3D spoiled gradient-echo (SPGR) sequence with dual-echo chemical shift-encoded water-fat separation,²⁶ TR, 5.5 ms; TE1, 1.15 ms; TE2, 2.3 ms; bandwidth, $\pm 63 \text{ kHz}$; slab volume, 40 cm (right to left [R/L]) $\times$ 32 cm (anterior to posterior [A/P]) $\times$ 24 cm (superior to inferior [S/I]); matrix, $224 \times 140 \times 48$; 15° flip angle; and partial k_z (0.75) acquisition for true spatial resolution of $1.8 \times 2.9 \times 5.0 \text{ mm}^3$ (interpolated to $1.1 \times 1.8 \times 2.5 \text{ mm}^3$ through zero-filling). Scan time was 23 s. No parallel imaging or B_0 sensitivity correction was performed to avoid spatially heterogeneous noise distributions and allow for absolute SNR measurements.²⁷

After the above-mentioned scans were performed using a fixed flip angle of 15° , the acquisition was repeated with flip angles from 10° to 50° at 5° increments to determine the optimal flip angle to maximize contrast between liver and muscle and between liver and the PV. To ensure that any observed signal differences were due to flip angle rather than slowly varying changes of the gadolinium concentration in the liver, we reversed the flip angle order in 6 of the 11 volunteers.

To determine the effects of dose on enhancement during the dynamic phase, we used a highly time-resolved T_1 -weighted 3D SPGR sequence. High spatial and temporal resolution were achieved using interleaved variable density (IVD) undersampling,²⁸ k-space corner-cutting,²⁹ and data-driven parallel imaging.³⁰ Autocalibration signal (ACS) lines were acquired at the center of the k-space only in the first time frame and then used for all 10 subsequent time frames acquired in the same breath-hold.³¹ Fat suppression was achieved using a dual-echo chemical shift-encoded water-fat separation.²⁶

Imaging parameters for the dynamic IVD sequence included: TR, 3.9 ms; TE1, 1.2 ms; TE2, 2.3 ms; bandwidth, $\pm 166.7 \text{ kHz}$; slab volume, 38 cm (R/L) $\times$ 34 cm (A/P) $\times$ 26 cm (S/I) matrix, $320 \times 202 \times 100$, 15° flip angle; and partial k_z (0.75) acquisition, $R = 2 \times 2$ data-driven parallel imaging acceleration (autocalibrating reconstruction for Cartesian imaging [ARC], GE Healthcare), for true spatial resolution of $1.2 \times 1.7 \times 2.6 \text{ mm}^3$ (interpolated to $0.74 \times 0.74 \times 1.3 \text{ mm}^3$ through zero-filling). Scan time was 24 s for 11 time frames with a temporal resolution of $\sim 2 \text{ s}$.

After obtaining the precontrast scan, dynamic-phase imaging was performed with the same pre-scan values to facilitate relative CNR calculations. The scan delay was fixed at 13 s after the beginning of the gadoxetic acid injection.

Data processing and analysis

Quantification of the STEAM datasets was performed using a custom script developed in Matlab (The Mathworks, Inc., Natick, MA, USA). The processing script performed joint fitting of all 28 spectra acquired with different TRs and TEs by accounting for T_2 decay and T_1 recovery. Each spectrum was fitted using Voigt line shapes and assuming a single peak for water signals and 6 peaks for fat signals.³² This processing resulted in estimates of water and fat T_1 and T_2 values as well as proton-density fat-fraction (PDFF) of the liver.

In addition, we calculated the estimated concentration of gadoxetic acid using:

$$\frac{1}{T_{1\text{Post}}} = C \cdot r_1 + \frac{1}{T_{1\text{Pre}}}, \quad [1]$$

in which c (mM) is the estimated concentration of gadoxetic acid in the liver; $T_{1\text{-Pre}}$ and $T_{1\text{-Post}}$ are the measured T_1 values of the liver before and after contrast injection; and r_1 is the relaxivity of gadoxetic acid. The longitudinal relaxivity, r_1 , is dependent on field strength and tissue type. However, no available reports describe the r_1 of the human liver at 3T. Previous studies reported $r_1 = 16.6$

$\text{mM}^{-1}\cdot\text{s}^{-1}$ for rat liver at 0.47T,³³ and $r_1 = 8.7 \text{ mM}^{-1}\cdot\text{s}^{-1}$ for plasma at 0.47T and $6.2 \text{ mM}^{-1}\cdot\text{s}^{-1}$ for plasma at 3T.³⁴ Considering all of these, we felt that the r_1 value for the liver at 0.47T, $16.6 \text{ mM}^{-1}\cdot\text{s}^{-1}$, was most likely the closest to that of the human liver at 3T.

We measured signals of MR images offline using open-source DICOM viewer software (OsiriX v. 5.8, Pixmeo, Geneva, Switzerland). A board-certified radiologist with fellowship training in abdominal MR imaging and 6 years of experience in diagnostic imaging drew regions of interest (ROI) in specific tissues of interest, copying the ROIs to identical positions in images obtained at different time points or with different flip angles. Slight manual corrections were made to ensure proper alignment with the relevant anatomy in the cases in which misregistration occurred from variability in breath-hold position. ROIs were placed in the liver (2 ROIs), muscle, and portal vein and in an artifact-free area outside the body. We used muscle as a surrogate for weakly enhancing liver lesions such as hypovascular metastasis.^{7,18}

We used the following equations to calculate the SNR of the liver, portal vein, and muscle:

$$\text{SNR} = \frac{SI_{\text{tissue}}}{SD_{\text{air}}} \times 0.7049, \quad [2]$$

in which SI_{tissue} is the average of signal intensity in tissues of interest and SD_{air} is the standard deviation of the signal of air measured in areas outside the body not compromised by artifacts. We used a correction factor of 0.7049 to account for differences in the behavior of the noise variance in the background region in magnitude images acquired with multi-channel coils.³⁵ We calculated the CNR to compare the contrast between the portal vein and muscle with the liver parenchyma (liver-to-portal vein and liver-to-muscle CNR). The CNR was calculated as the difference in SNR between the liver and the tissue of interest:

$$\text{CNR} = \text{SNR}_{\text{liver}} - \text{SNR}_{\text{tissue}}. \quad [3]$$

We placed ROIs in the hepatic artery, portal vein, and liver (2 ROIs) to evaluate the performance of the time-resolved IVD scan during the dynamic phase, used the average signal of the 2 liver ROIs in all subjects to minimize the effect of signal variations, and placed all ROIs equidistant to the anterior and posterior coils to account for the higher coil sensitivity for tissue closer to the coils. We measured the performance of the relative CNR (CNR_{rel}) in the dynamic phase of gadoxetic acid to determine the impact of contrast dose during the dynamic phase using:

$$\text{CNR}_{\text{rel}} = \frac{SI_{\text{vessels}} - SI_{\text{liver}}}{SI_{\text{Precontrast liver}}}, \quad [4]$$

in which SI_{vessels} is the signal intensity of the portal vein and hepatic artery and SI_{liver} is that of the liver during the dynamic phase. $SI_{\text{Precontrast liver}}$ is the signal intensity of the liver before contrast injection. Relative CNR was used to evaluate the performance of the time-resolved acquisition because accurate absolute SNR and CNR measurements are not possible when using parallel imaging.²⁷

Statistical analysis

All data are presented as the mean with 95% confidence intervals (95%CI) in all plots. We used paired t-test with Bonferroni correction to compare post-contrast (3, 10, and 20 min) T_1 and T_2 values of the liver with those values prior to contrast administration and paired t-tests to compare T_1 values of the liver, estimated contrast agent concentration in the liver, and SNR, CNR, and CNR_{rel} of 0.025- and 0.05-mmol/kg doses at each time point. We also used paired t-test to compare measurements with a dose of a 0.05 mmol/kg at a 10-min delay to measurements with a dose of 0.025 mmol/kg at a 20-min delay to explore the potential advantage of a higher dose for shortening the total scan time. A 2-sided t-test with P value less than 0.05 was considered statistically significant. All statistical tests were performed using Microsoft Excel 2010.

Results

T_1 and T_2 value and signal alteration at 3-, 10-, and 20-min delays

After injection of gadoxetic acid, the T_1 values decreased significantly ($P < 0.001$) from ~ 1000 ms before contrast administration to 284 ms with 0.025 mmol/kg and 220 ms with 0.05 mmol/kg. We observed significant changes in the T_1 value of the liver even 3 min after injection. The mean (95% CI) T_1 values were significantly lower for 0.05 mmol/kg than for 0.025 mmol/kg at all time points after injection of gadoxetic acid (all $P < 0.001$) (Fig. 1a). The mean (95% CI) T_1 values of the 0.05-mmol/kg dose compared to the 0.025-mmol/kg dose were: 293 ms (263–323 ms) versus 376 ms (344–408 ms) at 3 min; 248 ms (223–272 ms) versus 326 ms (297–356 ms) at 10 min; and 220 ms (199–241 ms) versus 284 ms (258–311 ms) at 20 min. Figure 1b shows the corresponding estimated concentrations of gadoxetic acid in the liver.

The average of the precontrast T_1 values over all subjects was slightly lower, although not significantly, between the first and second scans ($1063 \pm$

Fig. 1. T_2 values measured from magnetic resonance (MR) spectroscopy were slightly, though significantly ($P < 0.001$), shorter after than before contrast administration for both doses. After contrast administration, T_1 values were significantly shorter with 0.05 mmol/kg than 0.025 mmol/kg at all time points (3, 10, and 20 min, $P < 0.01$), but T_2 values were not ($P = 0.112$ to 0.183). Estimated concentrations of the contrast agent in the liver were also significantly higher using 0.05 mmol/kg at all time points. The estimated concentration of the contrast agent was calculated using the measured T_1 values of the liver and known r_1 value in the liver of rats at 0.47T ($16.6 \text{ mM}^{-1} \cdot \text{s}^{-1}$). The graphs show mean values with 95% confidence intervals.

171 ms versus 997 ± 159 ms, $P = 0.08$). This initially suggested that a very small amount of contrast may have remained in the liver in some subjects. For this reason, we plotted the precontrast T_1 values in Fig. 2. As the figure shows, precontrast T_1 values were lower for the first scan in half ($n = 5$) of the subjects and for the second scan in the other half ($n = 6$). However, we observed a relatively large difference in 2 subjects with lower T_1 values at the second scan that led to an overall decrease in the average of the precontrast T_1 values between the 2 scans. The intervals of the 2 examinations in the 2 subjects were 28 and 69 days.

Interestingly, the T_1 measured in enhanced liver tissue using 0.05 mmol/kg of gadoxetic acid at 10 min was significantly shorter than that for 0.025 mmol/kg at 20 min ($P < 0.001$). The estimated concentration of gadoxetic acid in the liver was also significantly higher at 10 min using the 0.05-mmol/kg dose than at 20 min using the 0.025-mmol/kg ($P = 0.008$, Fig. 1a).

T_2 values of the liver also decreased significantly, though just slightly ($P < 0.001$), after injection of gadoxetic acid from ~ 27 ms before contrast administration to ~ 25 with the 0.025-mmol/kg dose and ~ 23 ms with the 0.05-mmol/kg dose. Significant, though small, changes in the T_2 values of liver

were seen even at 3 min after injection. T_2 values did not differ significantly at any time point between the 2 doses of gadoxetic acid ($P = 0.112$ to 0.183) (Fig. 1a).

SNR performance in the hepatobiliary phase

The SNR of the liver during the hepatobiliary phase was significantly higher using 0.05 mmol/kg compared to 0.025 mmol/kg at all time points ($P < 0.001$; Figs. 3, 4). The SNR in the portal vein was also significantly higher using a 0.05-mmol/kg dose at 3 and 10 min ($P < 0.001$) and at 20 min ($P = 0.006$). There was no significant difference between the 2 doses for the SNR of muscle ($P = 0.581$ to 0.761). The CNR of the liver relative to the portal vein was significantly higher using 0.05 mmol/kg than 0.025 mmol/kg ($P = 0.002$ at 3 min; $P < 0.001$ at 10 and 20 min), and the CNR of the liver relative to muscle was significantly higher for 0.05 mmol/kg than 0.025 mmol/kg at all time points (all $P < 0.001$).

As observed with the T_1 measurements, the SNR of the liver ($P = 0.002$, Fig. 4) and CNR of the liver relative to muscle ($P = 0.006$, Fig. 4b) were also significantly higher at 10 min using 0.05 mmol/kg than at 20 min using 0.025 mmol/kg, whereas no significant difference was observed in the CNR of

Fig. 2. (a) We observed a small but insignificant difference between the average of precontrast T_1 values (1063 ± 171 ms versus 997 ± 159 ms, $P = 0.08$) between the first and second scans despite a relatively long washout period between scans (28 to 76 days, average 57 days). This initially suggested that a very small amount of contrast medium may have remained in the liver of some subjects. (b) However, the change between precontrast T_1 values in the subjects was highly variable and not related to the number of days between 2 scans. The relatively larger apparent change in T_1 values of 2 subjects may have skewed the average.

Fig. 3. Representative 3-dimensional (3D) gradient-echo T_1 -weighted images of the liver in a 32-year-old woman obtained 3, 10, and 20 min after injection of gadoxetic acid at 0.05 mmol/kg (bottom) and 0.025 mmol/kg (top). All images are shown with the same window width and window level. Images with a dose of 0.05 mmol/kg show higher signal-to-noise (SNR) and contrast-to-noise (CNR) ratios than those with 0.025 mmol/kg at every time point. Note: The CNR at 10 min using 0.05 mmol/kg was higher than that at 20 min with 0.025 mmol/kg. The CNR of the liver to portal vein (Liver-PV) and liver to muscle (Liver-muscle) are shown in the right bottom of each image.

Fig. 4. Absolute signal-to-noise ratio (SNR) and contrast-to-noise (CNR) performance for delayed T_1 -weighted imaging for 0.025- and 0.05-mmol/kg doses of gadoxetic acid. SNRs of the liver were significantly higher using 0.05 mmol/kg than 0.025 mmol/kg at every time point ($P < 0.001$). SNRs of the portal vein were also higher with 0.05 mmol/kg (left, $P < 0.006$) but not for muscle (right, $P = 0.581$ to 0.761). Both liver-to-portal vein and liver-to-muscle CNRs were significantly higher with 0.05 mmol/kg than 0.025 mmol/kg at every time point ($P < 0.002$). The graph plots are mean value with 95% confidence interval. SNR values of the liver are shown in both graphs as solid bars.

Fig. 5. Example images acquired as part of the flip angle optimization. The highest contrast-to-noise ratio (CNR) values were observed in a hepatobiliary-phase image with 20° for both 0.05 mmol/kg (bottom) and 0.025 mmol/kg (top) in the same volunteer shown in Fig. 3. Liver-to-portal vein (L-PV) and liver-to-muscle (L-muscle) CNR are shown in the right bottom of each image.

the liver relative to the portal vein ($P = 0.751$, Fig. 4a).

Flip angle optimization in the hepatobiliary phase

The SNRs in the portal vein and muscle were highest with the lowest flip angle (10°) for both doses, whereas the SNR in the liver was highest at 15° for the 0.025-mmol/kg dose and at 20° for the 0.05-mmol/kg dose (Figs. 5, 6). The CNR of the

liver to the portal vein was highest at a flip angle of 20° for both doses, 0.025 mmol/kg (mean [SD], 168^{32}) and 0.05 mmol/kg (232^{31}). The CNR of the liver to muscle was also the highest at 20° for both doses, 0.025 mmol/kg (174^{25}) and 0.05 mmol/kg (262^{52}) (Fig. 6).

Comparing the 2 doses, the CNRs of both the liver to the portal vein and the liver to muscle were significantly higher using the 0.05-mmol/kg dose

Fig. 6. Flip angle optimization results for the delayed hepatobiliary phase for 0.025 mmol/kg and 0.05 mmol/kg of gadoxetic acid. Among the hepatobiliary-phase images (acquired at 20 min) with various flip angles, the highest signal-to-noise ratios (SNR) of the liver (left) were obtained at 20° for 0.05 mmol/kg and 15° with 0.025 mmol/kg, whereas the SNR of the portal vein (center) and muscle (right) were the highest at the lowest flip angle (10°) for both doses. The contrast-to-noise ratio (CNR) of both the liver to portal vein and liver to muscle were highest at a flip angle of 20° using either dose. CNR values were significantly higher with 0.05 mmol/kg compared to 0.025 mmol/kg for all flip angles ($P < 0.013$) except for that for the liver to portal vein at the lowest flip angle of 10° ($P = 0.161$).

compared to 0.025 mmol/kg for all flip angles (all $P < 0.013$) except for CNR of liver to portal vein at the lowest flip angle of 10° ($P = 0.161$) (Fig. 6).

Contrast enhancement of the vessels in the dynamic phase

During the dynamic phase, the CNR_{rel} of the hepatic artery to the liver was significantly higher using 0.05 mmol/kg compared to 0.025 mmol/kg at a 17- to 25-s delay, whereas the CNR_{rel} of the portal vein to the liver was highest at a 27- to 35-s delay (Fig. 7). These data show the significant increase of peak enhancement in both the hepatic artery and portal vein with 0.05 mmol/kg ($P < 0.035$) (Fig. 8).

Discussion

In this work, we have performed a systematic and objective comparison of 0.025-mmol/kg and 0.05-mmol/kg doses of gadoxetic acid as part of an intraindividual crossover study. Specifically, we performed MR relaxometry to measure both T_1 and T_2 values in the liver after the injection of the different doses of gadoxetic acid. Further, we quantified a significant improvement in SNR and CNR performance during both the dynamic and hepatobiliary phases by increasing the dose from 0.025 mmol/kg to 0.05 mmol/kg. We also performed flip angle optimization to determine the T_1 weighting that maximizes SNR and CNR performance in the hepatobiliary phase for both contrast doses.

Our results indicate that increasing the dose of gadoxetic acid significantly reduces the T_1 value of the liver and increases the SNR of the liver and CNR of the liver relative to the portal vein and muscle during the hepatobiliary phase. During the dynamic phase, enhancement was significantly higher in both the hepatic artery and portal vein with the 0.05-mmol/kg dose than with 0.025 mmol/kg. Further, we determined 20° as the optimal flip angle to maximize the CNR of the liver versus the portal vein/muscle for either dose protocol.

We also demonstrated that using the 0.05-mmol/kg dose of gadoxetic acid, the T_1 value of the liver at 10 min was shorter than that using 0.025 mmol/kg at 20 min. The CNR between the liver and muscle using 0.05 mmol/kg at 10 min was also higher than that using 0.025 mmol/kg at 20 min. Interestingly, the T_2 values of the liver during the hepatobiliary phase were only minimally (albeit statistically significantly) affected by gadoxetic acid in general and not affected by increasing the gadolinium dose. Several researchers have suggested that T_2 -weighted imaging can be performed after gadoxetic acid injection, before the 20-min hepatobiliary as a means to shorten the overall protocol time.^{36–39} Our T_2 measurements confirm that T_2 -weighted imaging after the injection of gadoxetic acid at either 0.025 or 0.05 mmol/kg should have minimal impact on T_2 contrast and can be used to shorten the overall protocol time.

Fig. 7. Examples of time-resolved dynamic contrast-enhanced magnetic resonance (MR) imaging using 0.025 mmol/kg (top) and 0.05 mmol/kg (bottom) in the same volunteer. Visible enhancement of all arteries and the kidneys was greater using 0.05 mmol/kg than with 0.025 mmol/kg. The time-resolved dynamic scan was performed with a temporal resolution of 2 s in one of the volunteers, a 51-year-old woman. Note a small hemangioma (arrows) also had visibly greater enhancement using 0.05 mmol/kg of gadoxetic acid. The hemangioma is also seen in earlier phases using the higher contrast dose. The window/level of the images are identical for all images.

P values by paired t-test (0.05 vs. 0.025 mmol/kg)

	Pre	15	17	19	21	23	25	27	29	31	33	35
HA-to-liver	0.101	0.719	0.035	0.019	0.031	0.009	0.035	0.119	0.423	0.702	0.975	0.761
PV-to-liver	0.805	0.928	0.843	0.865	0.540	0.346	0.056	0.013	0.008	0.007	0.025	0.025

Fig. 8. Time-intensity graph of the hepatic artery (left) and portal vein (right) showed significantly higher relative contrast-to-noise ratios (CNRs) against the liver with a 0.05-mmol/kg dose than with 0.025 mmol/kg at a 17- to 26-s delay for the hepatic artery and 29- to 36-s delay for the portal vein.

Enhancement of the vessels during the dynamic phase typically depends upon the concentration of the contrast agent in the vessels, which is affected by both dose and injection speed.^{40,41} The recommended dose of gadoxetic acid, 0.025 mmol/kg, and injection speed, ≤ 2 mL/s,⁴² would result in lower intravascular gadolinium concentration dur-

ing the dynamic phase than that obtained with conventional gadolinium-based contrast agents, which are typically administered with a dose of 0.1 mmol/kg at ~ 2 mL/s.⁴³ Even considering the higher r_1 value effect with gadoxetic acid, the contrast effect is less with 0.025 mmol/kg of gadoxetic acid than that obtained with conventional gadolinium-based

contrast agents.³⁴ Emerging evidence also demonstrates that 0.025 mmol/kg may be an insufficient dose for dynamic-phase imaging.^{18,44–46} Therefore, some radiologists prefer to use an undiluted fixed dose of 10 mL of gadoxetic acid independent of the patient's weight and thereby often exceed the recommended package insert dose.⁴⁷ As expected, we observed significantly greater arterial and portal enhancement in the early dynamic phase using a higher dose, which may support the use of doses exceeding 0.025 mmol/kg in clinical practice.

During the hepatobiliary phase, however, the impact of higher doses for liver-to-vessel and liver-to-lesion contrast have been controversial.¹⁷ A recent intraindividual comparison of 0.025 and 0.05 mmol/kg in patients with cirrhosis suggested significant improvement of liver-to-lesion (hepatocellular carcinoma) contrast in patients with decreased hepatic function (Child-Pugh class B disease) but not in patients with preserved hepatic function (Child-Pugh class A disease).²¹ A study performed in the early stages of gadoxetic acid development also showed that liver-to-lesion contrast during the hepatobiliary phase was not necessarily higher using 0.05 compared with 0.025 mmol/kg, although the degree of enhancement compared to precontrast images was consistently higher using 0.05 mmol/kg.¹⁶

Our estimates of gadoxetic acid concentration in the liver during the hepatobiliary phase (Fig. 1b) demonstrated that using 0.05 mmol/kg significantly increased but did not double the intrahepatic concentration. This can be explained by the ability of the hepatocytes to take up gadoxetic acid; according to Michaelis-Menten kinetics, a higher concentration of contrast agents results in their slower uptake ratio due to the limitation of receptor ability.⁴⁸ This speculation is complicated by the increased renal excretion that would be expected with higher contrast doses, but it is supported by the higher SNR of the portal vein with a higher dose. Additionally, we have demonstrated that the use of greater flip angles can improve SNR and CNR performance. It is possible that the previous study could not demonstrate a difference in lesion-to-liver CNRs between 0.025- and 0.05-mmol/kg doses because we used a suboptimal flip angle (12°).²¹ Further, the difference in results between the previous and current studies can be explained by the homogeneity of the study subjects; in this study, we assessed volunteers and not patients with cirrhosis and muscle rather than liver lesions as the object.

Acquisition of reliable hepatobiliary-phase images by gadoxetic acid-enhanced MR imaging requires a delay of approximately 20 min after the administration of the contrast medium. To shorten

the total protocol time, some researchers have suggested the equivalent diagnostic ability for the detection of liver lesions of hepatobiliary-phase images obtained after 10 min.^{49,50} Despite the comparable results of visual assessment, quantitative results have shown lower contrast between the liver and lesion at 10 than 20 min.⁵¹ Our results suggest that the use of 0.05 mmol/kg resulted in higher SNR and CNR at 10 min than did 0.025 mmol/kg at 20 min. Considering these results, the use of a 10-min delay with an increased dose of gadoxetic acid can be justified both quantitatively and qualitatively. Shortening the overall protocol time may at least partially mitigate the increased cost of using a higher contrast dose.

This study had several limitations. First, our subjects were all healthy volunteers without liver disease. Because the pharmacokinetics of gadoxetic acid could be different in patients, especially those with cirrhosis,⁵² the clinical utility of 0.05 mmol/kg compared to 0.025 mmol/kg should be further evaluated in patients with focal liver lesions and/or diffuse liver disease. However, before studying patients with disease, it is important to optimize acquisition parameters and measure the range of normal relaxometry values in normal subjects.

An additional limitation is that we used the signal intensity of muscle as a surrogate for that of hypovascular intrahepatic lesions like metastases from colorectal carcinoma. According to this assumption, the advantage of a 0.05-mmol/kg over 0.025-mmol/kg dose was quantitatively obvious. However, we cannot conclude a definitive advantage of the 0.05-mmol/kg dose of gadoxetic acid until the diagnostic ability of the 2 doses is compared in patients with focal liver lesions.

Further, during the dynamic phase, we employed high temporal resolution (2 s) to show the time-to-intensity curve of each vessel. This sequence was well validated in previous studies.³¹ However, the view-sharing technique used in this sequence can introduce temporal blurring that can affect signal measurement. Nevertheless, we believe that the relative signal intensity among the phases within a single scan offers a reasonable comparison.

Estimation of the concentration in the liver has important limitations. The longitudinal relaxivity (r_1) of a contrast agent may vary between different field strengths, solvents, or tissues. The resulting concentration estimates in our study were calculated assuming the known r_1 in the liver of rats at 0.47T, which may lead to inaccurate estimates of concentration in the human liver at 3T. In any case, the relative estimates of concentration remain meaningful because the r_1 value is a simple scaling co-

efficient.

Finally, the order of injection of the 2 different doses of gadoxetic acid was the same in all subjects, 0.025 mmol/kg followed by 0.05 mmol/kg a month or more later. Although precontrast T_1 values did not differ significantly between the 2 doses of gadoxetic acid, that of the second scan (0.05 mmol/kg) was slightly lower than that of the first (Fig. 1). If we assume that the precontrast liver at the first scan had 0 mM of gadoxetic acid, the estimated residual concentration of gadoxetic acid in the liver at the second scan would be approximately 0.004 ± 0.007 mM, assuming $r_1 = 16.6 \text{ mM}^{-1}\text{s}^{-1}$. Considering the estimated concentration of gadoxetic acid in the liver between the 2 doses at 3 min (0.025 and 0.05 mmol/kg, 0.10 and 0.14 mM), 10 min (0.13 and 0.19 mM), and 20 min (0.16 and 0.22 mM), the effect of the precontrast T_1 value is probably not very important. Furthermore, previous research suggests that residual gadoxetic acid in the liver 7 days after administration was $\sim 0.17\%$ of the total dose for rats and 0.28 to 0.52% of the total dose for monkeys.³³ Those findings indicate that the effect of residual contrast in the liver at 4 weeks should be minimal compared to the further T_1 shortening effect achieved by doubling (200%) the dose.

In conclusion, our study has demonstrated that 0.05 mmol/kg of gadoxetic acid for hepatobiliary-phase MR imaging yields shorter T_1 values, higher SNR of the liver, and higher CNR of the liver relative to the portal vein and muscle than those with 0.025 mmol/kg. The higher dose also results in higher arterial and portal venous enhancement during the dynamic phase. These quantitative results also support the use of gadoxetic acid-enhanced hepatobiliary-phase images at 10 min instead of 20 min when using a 0.05-mmol/kg dose. Further work is needed to confirm these results in patients with diffuse liver disease and focal liver lesions.

Acknowledgement

This study was supported by research and development faculty research funding of the Department of Radiology of the University of Wisconsin.

The authors wish to acknowledge GE Healthcare, who provides research support to the University of Wisconsin.

References

- Okigawa T, Utsunomiya D, Tajiri S, et al. Incidence and severity of acute adverse reactions to four different gadolinium-based MR contrast agents. *Magn Reson Med Sci* 2014; 13:1–6.
- Frydrychowicz A, Lubner MG, Brown JJ, et al. Hepatobiliary MR imaging with gadolinium-based contrast agents. *J Magn Reson Imaging* 2012; 35:492–511.
- Zech CJ, Bartolozzi C, Bioulac-Sage P, et al. Consensus report of the Fifth International Forum for Liver MRI. *AJR Am J Roentgenol* 2013; 201:97–107.
- Park MJ, Kim YK, Lee MW, et al. Small hepatocellular carcinomas: improved sensitivity by combining gadoxetic acid-enhanced and diffusion-weighted MR imaging patterns. *Radiology* 2012; 264:761–770.
- Kim YK, Lee MW, Lee WJ, et al. Diagnostic accuracy and sensitivity of diffusion-weighted and of gadoxetic acid-enhanced 3-T MR imaging alone or in combination in the detection of small liver metastasis (≤ 1.5 cm in diameter). *Invest Radiol* 2012; 47:159–166.
- Grazioli L, Bondioni MP, Haradome H, et al. Hepatocellular adenoma and focal nodular hyperplasia: value of gadoxetic acid-enhanced MR imaging in differential diagnosis. *Radiology* 2012; 262:520–529.
- Nagle SK, Busse RF, Brau AC, et al. High resolution navigated three-dimensional T(1)-weighted hepatobiliary MRI using gadoxetic acid optimized for 1.5 Tesla. *J Magn Reson Imaging* 2012; 36:890–899.
- Van Beers BE, Pastor CM, Hussain HK. Primovist, Eovist: what to expect? *J Hepatol* 2012; 57:421–429.
- Motosugi U, Ichikawa T, Araki T. Rules, roles, and room for discussion in gadoxetic acid-enhanced magnetic resonance liver imaging: current knowledge and future challenges. *Magn Reson Med Sci* 2013; 12:161–175.
- Motosugi U, Bannas P, Sano K, Reeder SB. Hepatobiliary MR contrast agents in hypovascular hepatocellular carcinoma. *J Magn Reson Imaging* 2015; 41:251–265.
- Joishi D, Ueno A, Tanimoto A, et al. Natural course of hypovascular nodules detected on gadoxetic acid-enhanced MR imaging: presence of fat is a risk factor for hypervascularization. *Magn Reson Med Sci* 2013; 12:281–287.
- Motosugi U, Ichikawa T, Morisaka H, et al. Detection of pancreatic carcinoma and liver metastases with gadoxetic acid-enhanced MR imaging: comparison with contrast-enhanced multi-detector row CT. *Radiology* 2011; 260:446–453.
- Goshima S, Kanematsu M, Watanabe H, et al. Gd-EOB-DTPA-enhanced MR imaging: prediction of hepatic fibrosis stages using liver contrast enhancement index and liver-to-spleen volumetric ratio. *J Magn Reson Imaging* 2012; 36:1148–1153.
- Yamada A, Hara T, Li F, et al. Quantitative evaluation of liver function with use of gadoxetate disodium-enhanced MR imaging. *Radiology* 2011; 260:727–733.
- Reeder SB. Gadolinium-based contrast agents: What

- does “single-dose” mean anymore? *J Magn Reson Imaging* 2014; 39:1343–1345.
16. Vogl TJ, Kümmel S, Hammerstingl R, et al. Liver tumors: comparison of MR imaging with Gd-EOB-DTPA and Gd-DTPA. *Radiology* 1996; 200:59–67.
 17. Reimer P, Rummeny EJ, Shamsi K, et al. Phase II clinical evaluation of Gd-EOB-DTPA: dose, safety aspects, and pulse sequence. *Radiology* 1996; 199: 177–183.
 18. Frydrychowicz A, Nagle SK, D’Souza SL, Vigen KK, Reeder SB. Optimized high-resolution contrast-enhanced hepatobiliary imaging at 3 tesla: a cross-over comparison of gadobenate dimeglumine and gadoxetic acid. *J Magn Reson Imaging* 2011; 34: 585–594.
 19. Choi JW, Lee JM, Kim SJ, et al. Hepatocellular carcinoma: imaging patterns on gadoxetic acid-enhanced MR Images and their value as an imaging biomarker. *Radiology* 2013; 267:776–786.
 20. Pietryga JA, Burke LM, Marin D, Jaffe TA, Bashir MR. Respiratory motion artifact affecting hepatic arterial phase imaging with gadoxetate disodium: examination recovery with a multiple arterial phase acquisition. *Radiology* 2014; 271:426–434.
 21. Motosugi U, Ichikawa T, Sano K, et al. Double-dose gadoxetic acid-enhanced magnetic resonance imaging in patients with chronic liver disease. *Invest Radiol* 2011; 46:141–145.
 22. Bannas P, Motosugi U, Hernando D, Rahimi MS, Holmes JH, Reeder SB. Combined gadoxetic acid and gadofosveset enhanced liver MRI: a feasibility and parameter optimization study. *Magn Reson Med* 2015 Feb 3; doi:10.1002/mrm.25554. [Epub ahead of print]
 23. Bae KT. Intravenous contrast medium administration and scan timing at CT: considerations and approaches. *Radiology* 2010; 256:32–61.
 24. Hamilton G, Middleton M, Sirlin C. *In vivo* liver 1H MRS measurement of PDFF, and T₁ and T₂ of water and fat, in a single breath-hold with multiple TRs and TEs. Proceedings of the 21st Annual Meeting of ISMRM. Salt Lake City, 2013; 1517.
 25. Hamilton G, Ma J, Cunha G, Sirlin C. Effect of gadolinium-based contrast agent on the relaxation properties of water and fat in human liver as measured *in vivo* by 1H MRS. Proceedings of the 21st Annual Meeting of ISMRM. Salt Lake City, 2013; 1516.
 26. Ma J. Breath-hold water and fat imaging using a dual-echo two-point Dixon technique with an efficient and robust phase-correction algorithm. *Magn Reson Med* 2004; 52:415–419.
 27. Reeder SB, Wintersperger BJ, Dietrich O, et al. Practical approaches to the evaluation of signal-to-noise ratio performance with parallel imaging: application with cardiac imaging and a 32-channel cardiac coil. *Magn Reson Med* 2005; 54:748–754.
 28. Wang K, Busse RF, Holmes JH, et al. Interleaved variable density sampling with a constrained parallel imaging reconstruction for dynamic contrast-enhanced MR angiography. *Magn Reson Med* 2011; 66:428–436.
 29. Bernstein MA, Fain SB, Riederer SJ. Effect of windowing and zero-filled reconstruction of MRI data on spatial resolution and acquisition strategy. *J Magn Reson Imaging* 2001; 14:270–280.
 30. Brau AC, Beatty PJ, Skare S, Bammer R. Comparison of reconstruction accuracy and efficiency among autocalibrating data-driven parallel imaging methods. *Magn Reson Med* 2008; 59:382–395.
 31. Salmani Rahimi M, Korosec FR, Wang K, et al. Combined dynamic contrast-enhanced liver MRI and MRA using interleaved variable density sampling. *Magn Reson Med* 2015; 73:973–983.
 32. Hernando D, Artz NS, Hamilton G, Roldan-Alzate A, Reeder S. Fully automated processing of multi-echo spectroscopy data for liver fat quantification. Proceedings of the 22nd Annual Meeting of ISMRM. Milan, Italy; 2014; 2884.
 33. Schuhmann-Giampieri G, Schmitt-Willich H, Press WR, Negishi C, Weinmann HJ, Speck U. Preclinical evaluation of Gd-EOB-DTPA as a contrast agent in MR imaging of the hepatobiliary system. *Radiology* 1992; 183:59–64.
 34. Rohrer M, Bauer H, Mintonovitch J, Requardt M, Weinmann HJ. Comparison of magnetic properties of MRI contrast media solutions at different magnetic field strengths. *Invest Radiol* 2005; 40:715–724.
 35. Dietrich O, Raya JG, Reeder SB, Ingrisch M, Reiser MF, Schoenberg SO. Influence of multichannel combination, parallel imaging and other reconstruction techniques on MRI noise characteristics. *Magn Reson Imaging* 2008; 26:754–762.
 36. Muhi A, Ichikawa T, Motosugi U, Sou H, Sano K, Araki T. Diffusion- and T₂-weighted MR imaging of the liver: effect of intravenous administration of gadoxetic acid disodium. *Magn Reson Med Sci* 2012; 11:185–191.
 37. Tamada T, Ito K, Yoshida K, et al. T₂-weighted magnetic resonance imaging of the liver: evaluation of the effect in signal intensity after Gd-EOB-DTPA enhancement. *J Comput Assist Tomogr* 2010; 34:182–186.
 38. Saito K, Araki Y, Park J, et al. Effect of Gd-EOB-DTPA on T₂-weighted and diffusion-weighted images for the diagnosis of hepatocellular carcinoma. *J Magn Reson Imaging* 2010; 32:229–234.
 39. Choi JS, Kim MJ, Choi JY, Park MS, Lim JS, Kim KW. Diffusion-weighted MR imaging of liver on 3.0-Tesla system: effect of intravenous administration of gadoxetic acid disodium. *Eur Radiol* 2010; 20:1052–1060.
 40. Heverhagen JT, Wright CL, Schmalbrock P, Knopp MV. Dose comparison of single versus double dose in contrast-enhanced magnetic resonance angiography of the renal arteries: intra-individual cross-over blinded trial using Gd-DTPA. *Eur Radiol* 2009; 19: 67–72.

41. Froehlich JM, Unterweger M, Kubik-Huch RA. Single- vs. double-dose in contrast-enhanced magnetic resonance angiography of the carotid arteries. *J Magn Reson Imaging* 2007; 26:1173–1174, author reply 1174.
 42. Tanimoto A, Lee JM, Murakami T, Huppertz A, Kudo M, Grazioli L. Consensus report of the 2nd International Forum for Liver MRI. *Eur Radiol* 2009; 19 Suppl 5:S975–S989.
 43. Semelka RC, Helmberger TK. Contrast agents for MR imaging of the liver. *Radiology* 2001; 218:27–38.
 44. Brismar TB, Dahlstrom N, Edsberg N, Persson A, Smedby O, Albiin N. Liver vessel enhancement by Gd-BOPTA and Gd-EOB-DTPA: a comparison in healthy volunteers. *Acta Radiol* 2009; 50:709–715.
 45. Runge VM. A comparison of two MR hepatobiliary gadolinium chelates: Gd-BOPTA and Gd-EOB-DTPA. *J Comput Assist Tomogr* 1998; 22:643–650.
 46. Feuerlein S, Gupta RT, Boll DT, Merkle EM. Hepatocellular MR contrast agents: enhancement characteristics of liver parenchyma and portal vein after administration of gadoxetic acid in comparison to gadobenate dimeglumine. *Eur J Radiol* 2012; 81: 2037–2041.
 47. Ringe KI, Husarik DB, Sirlin CB, Merkle EM. Gadoxetate disodium-enhanced MRI of the liver: part 1, protocol optimization and lesion appearance in the noncirrhotic liver. *AJR Am J Roentgenol* 2010; 195: 13–28.
 48. Schuhmann-Giampieri G. Nonlinear pharmacokinetic modeling of a gadolinium chelate used as a liver-specific contrast agent for magnetic resonance imaging. *Arzneimittelforschung* 1993; 43:1020–1024.
 49. Jeong HT, Kim MJ, Park MS, et al. Detection of liver metastases using gadoxetic-enhanced dynamic and 10- and 20-minute delayed phase MR imaging. *J Magn Reson Imaging* 2012; 35:635–643.
 50. Motosugi U, Ichikawa T, Tominaga L, et al. Delay before the hepatocyte phase of Gd-EOB-DTPA-enhanced MR imaging: is it possible to shorten the examination time? *Eur Radiol* 2009; 19:2623–2629.
 51. Sofue K, Tsurusaki M, Tokue H, Arai Y, Sugimura K. Gd-EOB-DTPA-enhanced 3.0 T MR imaging: quantitative and qualitative comparison of hepatocyte-phase images obtained 10 min and 20 min after injection for the detection of liver metastases from colorectal carcinoma. *Eur Radiol* 2011; 21:2336–2343.
 52. Tsuda N, Matsui O. Cirrhotic rat liver: reference to transporter activity and morphologic changes in bile canaliculi–gadoxetic acid-enhanced MR imaging. *Radiology* 2010; 256:767–773.
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