MR Tracking of Iron-labeled Glass Radioembolization Microspheres during Transcatheter Delivery to Rabbit VX2 Liver Tumors: Feasibility Study¹

Twinkle Gupta, MS
Sumeet Virmani, MD
Tod M. Neidt, MS
Barbara Szolc-Kowalska, MD
Kent T. Sato, MD
Robert K. Ryu, MD
Robert J. Lewandowski, MD
Vanessa L. Gates, MS
Gayle E. Woloschak, PhD
Riad Salem, MD, MBA
Reed A. Omary, MD, MS
Andrew C. Larson, PhD

Purpose:

To prospectively test the hypothesis that iron labeling of radioembolization microspheres permits their visualization by using magnetic resonance (MR) imaging for in vivo tracking during transcatheter delivery to liver tumors.

Materials and Methods:

All experiments were approved by the Institutional Animal Care and Use Committee. Phantom studies were performed to quantify microsphere relaxivity and volume susceptibility properties and compare image contrast patterns resulting from aggregate deposition of unlabeled and iron-labeled microspheres. In seven rabbits in which nine VX2 liver tumors were implanted, T2*-weighted gradient-echo (GRE) MR images with negative image contrast (NC), white-marker (WM) GRE images with positive image contrast (PC), and on-resonance water-suppression turbo spin-echo (SE) images with PC were obtained before and after catheter-directed administration of microspheres into the hepatic artery. During each injection, serial GRE acquisitions were performed for real-time visualization of microsphere delivery. Contrast-to-noise ratios (CNRs) were measured between regions of microsphere accumulation and regions of normal liver parenchyma that demonstrated no apparent microsphere accumulation. Pre- and postinjection CNR measurements at identical spatial positions were compared by using paired *t* test ($\alpha = .05$).

Results:

Conventional microspheres did not produce detectable image contrast in phantoms. Iron-labeled microspheres produced susceptibility-induced dipole patterns with spatial extent of image contrast increasing with increasing microsphere dose. Real-time image series depicted both preferential delivery to tumor tissues and nontargeted delivery to adjacent organs. T2*-weighted GRE, WM GRE, and on-resonance water-suppression turbo SE each permitted in vivo visualization of the microsphere deposition, with postinjection CNR values (mean, 14.29 ± 3.98 [standard deviation], 1.87 ± 0.93 , and 19.30 ± 8.72 , respectively) significantly greater than corresponding preinjection CNR values (mean, 2.02 ± 4.65 , 0.02 ± 0.27 , 0.85 ± 2.65 , respectively) ($P < .05$).

Conclusion:

Microsphere tracking during radioembolization may permit real-time verification of delivery and detection of extrahepatic shunting.

¹ From the Department of Radiology, Northwestern University, 448 E Ontario St, Suite 700, Chicago, IL 60611. Received November 19, 2007; revision requested February 5, 2008; revision received February 22; accepted March 25; final version accepted April 7. Address correspondence to A.C.L. (e-mail: a-larson@northwestern.edu).

Radioembolization with glass yttrium 90 (^{90}Y) microspheres is a promising form of internal radiation therapy for hepatocellular carcinoma (1). Compared with external-beam radiation, selective intraarterial injection of ^{90}Y microspheres increases the regional radiation dose to targeted tumors, with minimal toxic effects to the normal liver. Researchers have established that this innovative therapy offers a safe, effective treatment option to a broad spectrum of liver cancer patients (2).

Radioembolization microspheres are administered through catheter-directed injections into hepatic arteries proximal to the targeted liver tumors (3–5). Similar to transcatheter arterial chemoembolization, radioembolization relies on differences in arterial and portal blood supplies between liver tumors and normal liver parenchyma for preferential delivery of microspheres to the targeted tumors (6).

Given the propensity for arterial variants and arteriovenous shunting, prior to microsphere infusion, pretreatment procedures are necessary to optimize catheter placement, eliminate vascular reflux pathways to adjacent organs, and estimate both lung shunt fraction and targeted liver volumes for radiation dosimetry. First, conventional abdominal angiography with selective celiac and superior mesenteric catheterization is performed to evaluate vascular anatomy and blood flow dynamics for optimal catheter placement (5). Technetium 99m ($^{99\text{m}}\text{Tc}$) macroaggregated albumin (MAA) is injected through the catheter, and subsequent single photon emission computed

tomographic (SPECT) techniques are used to estimate lung shunt fraction and detect gastrointestinal shunting. Radiation dosimetry calculations are based on the mass of the targeted lobar or segmental liver volume, the lung shunt fraction, and the overall activity to be delivered by the injected ^{90}Y microspheres.

Preprocedural scans obtained with SPECT and $^{99\text{m}}\text{Tc}$ -MAA offer rather poor approximations of intraprocedural microsphere biodistribution in the liver. Direct in vivo visualization of glass microspheres would permit immediate verification of microsphere delivery at the time of infusion and detection of extrahepatic shunting. Alternatively, similar to scans obtained with SPECT and $^{99\text{m}}\text{Tc}$ -MAA, a nonradioactive tracer dose of iron-labeled microspheres could potentially be injected to predict extrahepatic shunting and estimate biodistribution prior to injection of radioactive microspheres. Unlike $^{99\text{m}}\text{Tc}$ -MAA particles, this tracer dose would have exactly the same composition, physical characteristics, and, hence, biodistribution as the therapeutic radioactive dose, thereby providing more accurate estimates of extrahepatic shunting and biodistribution. Investigators in previous studies using holmium microspheres for internal radiation therapy exploited the intrinsic paramagnetic properties of holmium 166 (^{166}Ho) to generate T2*-weighted contrast for magnetic resonance (MR) imaging of intrahepatic biodistribution (7,8). However, unlike ^{166}Ho microspheres, glass ^{90}Y microspheres are only weakly paramagnetic. The success of previous MR imaging studies

with ^{166}Ho and the recent proliferation of iron-labeled stem cell-tracking techniques (9,10) led us to investigate the feasibility of labeling glass microspheres with superparamagnetic iron oxides for in vivo visualization with MR imaging.

The purpose of our study was to prospectively test the hypothesis that iron labeling of radioembolization microspheres permits their visualization by using MR imaging for in vivo tracking during transcatheter delivery to liver tumors.

Materials and Methods

Iron-labeled Glass Microspheres

Conventional unlabeled glass microspheres produce insufficient perturbations of the local magnetic field for visualization of paramagnetic dephasing effects at clinical MR imaging field strengths. Therefore, to permit visualization, we proposed labeling glass microspheres with iron oxide particles. For an experimental iron-labeled ^{90}Y microsphere surrogate, we used glass ceramic microspheres composed of Fe_3O_4 nanocrystals dispersed throughout a silicate glass (MO-SCI,

Advances in Knowledge

- Iron labeling permits visualization of glass radioembolization microspheres with MR imaging.
- Tracking with MR imaging permits real-time verification of microsphere delivery to liver tumors.
- Positive-image-contrast and negative-image-contrast MR imaging methods depict intrahepatic microsphere deposition and nontargeted delivery to the gastrohepatic trunk.

Implications for Patient Care

- We demonstrated that MR imaging permits visualization of iron-labeled glass microspheres during transcatheter delivery to liver tumors.
- Microsphere tracking may permit real-time verification of delivery, detection of extrahepatic shunting, and improved radiation dosimetry.

Published online before print

10.1148/radiol.2491072027

Radiology 2008; 249:845–854

Abbreviations:

AMD = aggregate microsphere deposition
CNR = contrast-to-noise ratio
FOV = field of view
GRE = gradient echo
MAA = macroaggregated albumin
NC = negative image contrast
PC = positive image contrast
ROI = region of interest
SE = spin echo
WM = white marker

Author contributions:

Guarantor of integrity of entire study, A.C.L.; study concepts/study design or data acquisition or data analysis/interpretation, all authors; manuscript drafting or manuscript revision for important intellectual content, all authors; manuscript final version approval, all authors; literature research, T.G.; experimental studies, all authors; statistical analysis, T.G.; and manuscript editing, T.M.N.

Authors stated no financial relationship to disclose.

Rolla, Mo). These glass ceramic microspheres were produced from a homogeneous iron rare earth silicate glass that contained 10 atom percent ionic iron as Fe^{2+} and Fe^{3+} (T.M.N.). The resulting microspheres were between 20 and 40 μm in diameter and were heat treated to precipitate the Fe_3O_4 nanocrystals (Fig 1).

Phantom Preparation

Two separate phantom models were constructed for this study. First, six aggregate microsphere deposition (AMD) phantoms were constructed by an author (T.G., with 2 years of experience). Of these AMD phantoms, five contained increasing doses of our iron-labeled glass microspheres (2.5, 5, 10, 20, and 40 mg, corresponding to samples of roughly 125 000, 250 000, 500 000, 1 000 000, and 2 000 000 microspheres, respectively) and one contained 40 mg (2 000 000 microspheres) of unlabeled glass microspheres. Each AMD phantom was prepared by dissolving 20 g of agarose powder and 30 mg of $\text{MnCl}_2 \cdot \text{H}_2\text{O}$ in 1 L of deionized water. $\text{MnCl}_2 \cdot \text{H}_2\text{O}$ was added to mimic relaxation properties of liver tissue. The agarose suspension was separated into six equal portions that were poured into 1-L plastic containers. A pipette tip was used to create a narrow 1-cm well at the center of each agar block. Six microsphere samples were then added to each well and immediately covered with an additional layer of agarose. The AMD phantom model was used to demonstrate the relationship between dose and spatial extent of image contrast and the lack of detectable image contrast around unlabeled microspheres.

A second phantom series was also created (five vials, 50-mL total volume for each vial) with increasing concentrations of homogeneously distributed microspheres (0, 0.05, 0.1, 0.2, and 0.4 mg/mL, corresponding to 0, 2500, 5000, 10 000 and 20 000 microspheres per milliliter) within the $\text{MnCl}_2 \cdot \text{H}_2\text{O}$ doped agarose suspension described previously. This five-vial phantom series was used to perform relaxometry and susceptibility measurements for the iron-labeled microspheres.

Animal Model

Our institutional Animal Care and Use Committee approved all experiments. Seven New Zealand white rabbits weighing 4–5 kg were used in these experiments. The VX2 tumor model was used because the VX2 tumor blood supply is almost entirely from the hepatic artery, similar to the blood supply of human hepatocellular carcinoma, and rabbit hepatic arteries are sufficiently large to permit catheterization (11). VX2 cells were initially grown in the hind limb of a donor rabbit. Tumor portions approximately 2 mm in diameter were harvested and implanted in the left lobe of the liver in the seven rabbits in a minilaparotomy procedure performed by an author (S.V., with 2 years of experience), thus inducing liver tumors in 14–28 days. In seven rabbits, nine VX2 liver tumors were grown (size range, 1.5–3.0 cm in diameter). During imaging procedures, rabbits were anesthetized with intramuscularly administered ketamine (Ketaset; Fort Dodge Animal Health, Fort Dodge, Iowa), and xylazine (AnaSed Injection; Lloyd Laboratories, Shenandoah, Iowa), followed by inhaled isoflurane (Isothesia; Abbott Laboratories, North Chicago, Ill).

X-ray Fluoroscopic Guidance

Each rabbit was catheterized with x-ray fluoroscopic guidance by using a C-arm unit (PowerMobil; Siemens Medical Solutions, Erlangen, Germany). Vascular access was achieved in the femoral ar-

tery through surgical cutdown. A 3-F vascular sheath was first placed, and a 2-F catheter was then inserted within this sheath. The 2-F catheter (JB-1; Cook, Bloomington, Ind) was advanced over a 0.014-inch-diameter guidewire into the targeted arteries. In six rabbits, the catheter was advanced into the hepatic artery, thereby targeting microsphere delivery to the liver. In one rabbit, the catheter was positioned in the proximal celiac artery to demonstrate the feasibility of detecting nontargeted delivery to the gastrophatic trunk. Conventional digital subtraction angiographic procedures were performed by an attending interventional radiologist (R.A.O., with >10 years of experience). The animals were then transferred to an MR imaging unit.

Transcatheter Intraarterial Microsphere Injections

After performing preinjection MR imaging, microspheres (20–40-mg microspheres per injection, corresponding to 1 000 000–2 000 000 spheres per injection) were suspended in 3-mL isotonic saline and administered with hand injection by an author (S.V.) through the 2-F catheter. Accounting for liver volume differences, the number of spheres, 1 000 000–2 000 000, used in the rabbit is comparable to that used clinically (12). Each injection was performed with the rabbit positioned inside the MR unit, thereby facilitating (a) acquisition of real-time image series for cinematic visualization

Figure 1: Left: Scanning electron micrograph of iron-labeled glass radioembolization microspheres. (Original magnification, $\times 500$.) Right: Histogram depicts microsphere size distribution, with 90% of microspheres within 20–40- μm -diameter range.

of microsphere delivery and (b) preservation of spatial registration for comparison of pre- and postinjection MR images.

MR Imaging

All MR imaging studies were performed by one investigator (T.G.) by using a 1.5-T clinical MR unit (Magnetom Sonata; Siemens Medical Solutions).

Relaxivity and susceptibility properties: phantom studies.—For quantification of microsphere r_2^* relaxivity and susceptibility properties, we used a conventional multi-gradient-echo (GRE) spin-echo (SE) sequence (13), with the following parameters: repetition time, 3000 msec; central SE echo time, 45 msec; echo train length, 19; echo spacing, 2 msec; bandwidth, 600 Hz/pixel; section thickness, 5 mm; and matrix, 256×256 . Each series of multi-GRE SE magnitude and phase images was used to measure R_2^* relaxation and volume susceptibility properties separately for each phantom vial (6). The transverse relaxation rate R_2 for each vial was determined by using a multi-echo SE sequence with the following parameters: 500/11, 50; bandwidth, 300 Hz/pixel; section thickness, 5 mm; and matrix, 256×256 .

AMD studies.—Both AMD phantom and animal studies were performed by using identical pulse sequence parameters. For all AMD phantom studies, image sections were centered at the position of microsphere deposition at orientations parallel to the static magnetic field. Rabbits were imaged in the head-first supine position by using a single-channel clinical head coil.

We used three previously established methods for generating MR image contrast on the basis of perturbations of the local magnetic field caused by strong susceptibility differences between neighboring materials (14–16). These methods included the following sequences: (a) conventional T_2^* -weighted GRE (14), (b) white-marker (WM) GRE (15), and (c) on-resonance water-suppression turbo SE (16). Local field gradients, arising from strong susceptibility differences between superparamagnetic particles and adja-

cent tissue, led to spatially dependent resonant-frequency offsets. For the so-called negative-image-contrast (NC) T_2^* -weighted GRE approach, these effects manifest as signal voids caused by spin dephasing across those voxels near the particles. For the positive-image-contrast (PC) WM GRE approach, a gradient imbalance is created by partial refocusing of the section-selection gradient, resulting in incomplete rephasing of on-resonance spins but partial rephasing of those spins within voxels near the particles. Within these voxels, the section-selection gradient imbalance is cancelled by the strong susceptibility-induced field gradients. Finally, for the on-resonance water-suppression turbo SE approach, a spectrally selective water-saturation pulse is applied to suppress signal from on-resonance spins while retaining signal from off-resonance spins within voxels located near the paramagnetic particles. Our on-resonance water-suppression turbo SE implementation was similar to the inversion-recovery with on-resonance water-suppression, IRON, approach described by Stuber et al (16), with the only exception being the lack of inversion-recovery pulses for fat suppression.

We acquired pre- and postinjection anatomic images with NC and PC at adjacent axial and coronal section positions. Anatomic images were acquired by using a T_2 -weighted turbo SE sequence with the following parameters: 3000/82; section thickness, 5 mm; bandwidth, 130 Hz/pixel; field of view (FOV), 200×100 mm; matrix, 256×256 ; turbo factor, seven; and number of signals acquired, four. Images with NC and PC were obtained by using the following common parameters: FOV, 200×100 mm; matrix, 256×256 ; and section thickness, 5 mm. T_2^* -weighted GRE and WM GRE specific parameters included the following: 85/4; flip angle, 25° ; bandwidth, 260 Hz/pixel; and section-selection refocusing for WM GRE, 0%. On-resonance water-suppression turbo SE specific parameters included the following: 3000/12; bandwidth, 260 Hz/pixel; spectrally selective water saturation bandwidth, 300 Hz; and turbo factor, 12.

For six rabbits with the catheter placed in the hepatic artery, real-time GRE images were obtained during catheter-directed infusion to depict dynamic distribution of the iron-labeled glass microspheres. Imaging parameters for real-time T_2^* -weighted GRE images included the following: 10/4; section thickness, 5 mm; number of sections, four, in either axial (three rabbits) or coronal (three rabbits) orientations; FOV, 200×100 mm; matrix, 256×256 ; flip angle, 25° ; bandwidth, 260 Hz/pixel; and temporal resolution, 1 frame per second. In the seventh rabbit with the catheter positioned in the celiac artery, T_2^* -weighted GRE images (85/4; flip angle, 25° ; bandwidth, 260 Hz/pixel) to cover the entire abdominal region were obtained.

Histopathologic Evaluation

Immediately after imaging studies, animals were euthanized, and each liver was harvested for subsequent histopathologic evaluation. Excised livers were fixed in 10% buffered formaldehyde solution and sliced at 5-mm intervals in coronal planes to correspond to the planes used during MR imaging. Segments from slices containing tumor nodules were embedded in paraffin for histopathologic examination. These segments were sliced in 35- μ m-thick sections and stained by using hematoxylin-eosin to identify tumor boundaries. A microscope (Axioskop; Zeiss MicroImaging, Thornwood, NY) and spectral camera (Nuance; CRI, Woburn, Mass) were used for hematoxylin-eosin-stained slide inspection (optical magnifications, $\times 2.5$ and $\times 10$).

At $\times 2.5$ magnification, the 30-mm FOV of the camera did not encompass entire slides, and, therefore, multiple contiguous FOV images were captured by manually traversing each slide. These limited FOV images were then “stitched” together for inspection of the complete slide (ImageAssembler; PanaVue, Quebec City, Quebec, Canada). Additional images of individual microsphere deposits were sampled at $\times 10$ magnification. Histopathologic images were examined (T.G.) to determine the location of microsphere deposits.

Data and Statistical Analysis

Relaxivity and susceptibility properties: phantom studies.—By using software (Image J 1.36b; Wayne Rasband, National Institutes of Health, Bethesda, Md), regions of interest (ROIs) were drawn to consistently measure mean signal intensity at the identical position within each phantom vial. SE signal intensity values were used to quantify the transverse R2 relaxation for each phantom vial by using least-squares fitting and assuming monoexponential signal decay (Or-

igin 7.0 SR0; Originlab, Northampton, Mass). Similarly, the signal intensity envelope generated from each multi-GRE SE image series (Fig 2a) was used to measure R2* relaxation for each phantom vial. Monoexponential fitting was performed separately for even and odd echoes with the statistical software just mentioned. These two separately fit parameters were averaged to provide the R2* relaxation rate for each vial. Microsphere r2 and r2* relaxivity properties were calculated, with the statistical

software, on the basis of a linear fit of R2 and R2* relaxation values to corresponding microsphere concentrations.

Multi-GRE SE phase images were used to determine the volume susceptibility of our microspheres. The phase difference between two subsequent echoes was used to calculate volume susceptibility per vial, given that $\Delta\phi = \gamma (-\Delta\chi) (TE_2 - TE_1) B_0/6$ for a cylinder perpendicular to the main magnetic field B_0 , where TE_2 and TE_1 are second and first echo times, respectively (17). Here, ϕ is the phase of

Figure 2: (a) Signal intensity-echo time curves for five-vial AMD phantom series (only even numbered echoes shown) as measured with multi-GRE SE sequence. (b) Transverse relaxation rates R2 and R2* at each iron-labeled glass microsphere concentration. (c) Images obtained with NC and T2*-weighted GRE (top), PC and WM GRE (middle), and PC and on-resonance water-suppression turbo SE (bottom) show AMD in phantom, with iron-labeled microsphere dose increasing from left to right. Arrow depicts direction of B_0 relative to phantom images on c. Iron-labeled microspheres produced characteristic dipole patterns, with the spatial extent of image contrast increasing with dose. These image contrast patterns were not observed for equivalent samples of unlabeled microspheres (far right). a.u. = Arbitrary units, ml = milliliters.

Figure 3

Figure 3: Serial microsphere accumulation in liver. At top left, axial T2-weighted turbo SE (T2W-TSE) anatomic image depicts liver tumor position (arrow) and distal branches of hepatic arteries (arrowheads). Iron-labeled microspheres were injected with catheter positioned in hepatic artery. Top middle and right and bottom: Real-time axial T2*-weighted GRE image series at 0–24 seconds after injection depicts microsphere delivery as dynamic reduction of signal intensity in regions of microsphere accumulation (arrows, bottom right, 24 seconds after injection). Microspheres distributed heterogeneously, with maximal deposition at tumor periphery and in distal branches of hepatic arteries. t = Time after injection.

Figure 4

Anatomic T2W-TSE

Figure 4: Serial microsphere accumulation in liver. Left: Coronal T2-weighted turbo SE anatomic image depicts liver tumor position (arrow). Iron-labeled microspheres were injected with catheter positioned in hepatic artery. Middle and right, top and bottom: Real-time coronal T2*-weighted GRE series at 0–18 seconds after injection depicts microsphere delivery as dynamic reduction of signal intensity in regions of microsphere accumulation. Microspheres distributed heterogeneously, with maximal deposition at tumor periphery. Significant deposition was also observed in distal branches of hepatic arteries (arrowheads on anatomic image). Keys are same as on Figure 3.

signal inside of the cylinder, γ is the gyromagnetic ratio, and $\Delta\chi$ is the difference in volume susceptibility. Volume susceptibility (parts per million per milligram per milliliter) was then calculated, with the statistical software, on the basis of a linear fit of $\Delta\chi$ values to corresponding microsphere concentrations.

AMD studies.—For images with NC and PC in coronal and axial orientations, we measured the relative contrast-to-noise ratio (CNR) in regions of iron-labeled microsphere accumulation. ROI selections on postinjection images (Image J 1.36b) were made with reference to corresponding images sampled immediately prior to infusion. Postinjection signal intensity changes were assumed to result from iron-labeled microsphere deposition. First, a peripheral ROI void of tissue or any apparent artifacts was selected (T.G.). The standard deviation of the signal intensity in this ROI was used as our relative noise estimate, or σ_n . Next, for each of these sections, we measured the mean signal intensity from an ROI in normal liver parenchyma, or S_b , that demonstrated no apparent microsphere deposition. Finally, we measured the mean signal intensity in each position of microsphere accumulation, or S_a , by using an ROI selected to encompass the entire contiguous region of either PC or NC. Each of these measurements was repeated on corresponding preinjection images by copying these ROI to the identical positions. From these measurements, we separately calculated pre- and postinjection relative CNR for each ROI as follows: $\text{CNR} = (S_a - S_b)/\sigma_n$.

Statistical methods.—We selected six to 12 ROIs (40–50 mm²) around regions of microsphere deposits for each rabbit ($n = 56$ total ROIs) for comparison of pre- and postinjection CNR values. Separately for each of the three techniques (T2*-weighted GRE, WM GRE, and on-resonance water-suppression turbo SE), we compared preinjection CNR measurements to postinjection CNR measurements by using the paired t test, with $\alpha = .05$, as calculated with the statistical software.

Results

Relaxivity and Susceptibility Properties: Phantom Studies

Multi-GRE SE signal envelopes for $R2^*$ relaxation measurement demonstrated increasing signal decay rates with increasing microsphere concentration (Fig 2a). On the basis of a linear fit to $R2$ and $R2^*$ relaxation measurements (Fig 2b), mean $r2^*$ and $r2$ relaxivities were determined to be $287.16 \text{ sec}^{-1} \cdot \text{mg}^{-1} \cdot \text{mL} \pm 22.89$ (standard deviation) ($r^2 = 0.99$) and $3.49 \text{ sec}^{-1} \cdot \text{mg}^{-1} \cdot \text{mL} \pm 0.18$ ($r^2 = 0.98$), respectively. $R2$ was largely independent of microsphere concentration, whereas $R2^*$ showed a strong linear concentration dependence. Mean volume susceptibility for our iron-labeled microspheres was $3.96 \text{ (ppm} \cdot \text{mg}^{-1})/\text{mL} \pm 0.24$ ($r^2 = 0.99$).

AMD Studies

For our aggregate microsphere deposition phantom, $T2^*$ -weighted GRE, WM GRE, and on-resonance water-suppression turbo SE images each produced characteristic dipole image contrast patterns (18), with spatial extent of the image contrast increasing with increased microsphere dose (Fig 2c).

Conventional unlabeled microspheres did not produce image contrast with either NC or PC approaches in the phantom. Real-time $T2^*$ -weighted GRE image series in axial and coronal orientations each depicted microsphere delivery as a time-dependent reduction of signal intensities within regions of microsphere deposition (Figs 3, 4). Microsphere accumulation was most apparent at the boundaries between tumor and normal liver parenchyma and within hepatic arteries. After celiac artery injection, axial and coronal $T2^*$ -weighted images clearly depicted extrahepatic microsphere delivery to the stomach (Fig 5). After celiac artery injection, no microsphere deposition was observed in the liver tumors.

Comparison of pre- and postinjection $T2^*$ -weighted GRE, WM GRE, and on-resonance water-suppression turbo SE images confirmed heterogeneous micro-

Figure 5

Figure 5: Detection of extrahepatic shunting. (a) Coronal and (d) axial $T2$ -weighted turbo SE anatomic images depict liver tumor position (arrow, a) and gallbladder (arrowhead, a). Horizontal dashed line on a depicts axial section position for d–f. For demonstration of nontargeted delivery, iron-labeled microspheres were injected with catheter positioned in celiac artery. (b) Pre- and (c) postinjection coronal $T2^*$ -weighted GRE images depict nontargeted iron-labeled microsphere delivery to stomach (arrows, c). (e) Pre- and (f) postinjection $T2^*$ -weighted GRE images at axial position (horizontal dashed line, a) depict microsphere delivery to stomach (arrows, f). Images also demonstrate feasibility of detecting microsphere deposition within tissues adjacent to air pockets in stomach (arrowheads, f). No microsphere deposition was observed in liver tumor.

sphere distribution with significant accumulation within tissues at tumor peripheries and within arterial blood vessels distal to the catheter position (Fig 6). In coronal orientations aligned parallel to the static magnetic field, regions of microsphere accumulation produced characteristic dipole image contrast patterns (18) in images with both NC and PC (Fig 6).

Histopathologic evaluation.—Images of histopathologic specimens stained with hematoxylin-eosin confirmed the deposition of iron-labeled microspheres within hepatic arteries and arterial branches of the portal triads near tumor peripheries (Fig 7). Scratch artifacts (dark slashes within the hematoxylin-eosin-stained slides) were commonly observed at these positions because microspheres greater than the selected $35\text{-}\mu\text{m}$ section thickness were crushed through the microtome during the slicing process.

Data and statistical analysis.— $T2^*$ -

weighted GRE, WM GRE, and on-resonance water-suppression turbo SE each depicted positions of iron-labeled microsphere deposition with significant mean increases from preinjection CNR of 2.02 ± 4.65 , 0.02 ± 0.27 , and 0.85 ± 2.65 , respectively, to mean postinjection CNR of 14.29 ± 3.98 , 1.87 ± 0.93 , and 19.30 ± 8.72 . A wide variability of microsphere deposition within different ROI led to a relatively large standard deviation within postinjection CNR measurements. Nonetheless, when compared pairwise to preinjection CNR levels, significant increases in CNR levels were observed for each of the $T2^*$ -weighted GRE, WM GRE, and on-resonance water-suppression turbo SE approaches (all $P < .05$).

Discussion

Radioembolization with glass ^{90}Y microspheres has proved to be an effective

palliative therapy for liver cancer. However, currently ^{90}Y microspheres cannot be visualized with conventional imaging methods. In our study, we investigated the feasibility of labeling glass microspheres with iron oxide particles for MR imaging visualization. By using a clinical MR unit, we demonstrated real-time visualization of iron-labeled microsphere delivery, as well as NC and PC approaches for depicting microsphere deposition, in liver tumors and hepatic blood vessels.

$^{99\text{m}}\text{Tc}$ -MAA particles have served as a useful analog for visualization of anticipated ^{90}Y microsphere biodistribution. However, while the majority of $^{99\text{m}}\text{Tc}$ -MAA particles are 30–90 μm , up to 10% may be well below this size range (19). Time-dependent breakdown of the $^{99\text{m}}\text{Tc}$ -MAA radioisotope and/or protein structure leads to reduction in particle size and free technetium. The latter factors each can lead to migration of particles through the normal capillary beds and potentially inaccurate predic-

tion of ^{90}Y microsphere biodistribution. Differences between $^{99\text{m}}\text{Tc}$ -MAA particles as opposed to spheres) and hemodynamic or catheter position changes between $^{99\text{m}}\text{Tc}$ -MAA studies and final ^{90}Y microsphere infusion each can lead to further discrepancies.

Preprocedural prediction and intra-procedural detection of extrahepatic shunting may be critical to limit toxic effects after radioembolization with ^{90}Y microspheres (2). Patients with sub-

Figure 6

Figure 6: Aggregate microsphere deposition at tumor boundaries. (a) Coronal T2-weighted turbo SE anatomic image depicts liver tumor position (arrow). Iron-labeled microspheres were injected with catheter positioned in hepatic artery. (b–g) Pre- and postinjection coronal (b, e) T2*-weighted images with NC, (c, f) WM GRE images with PC, and (d, g) on-resonance water-suppression turbo SE images with PC depict microsphere delivery to periphery of tumor. PC approach demonstrated suppression of baseline signal intensity in liver and subsequent positive signal at positions of microsphere deposition, whereas NC approach produced signal voids at microsphere positions. Similar to earlier phantom studies, these coronal NC and PC images parallel to the main static field produced characteristic dipole patterns at positions of microsphere deposition.

Figure 7

Figure 7: Histopathologic evaluation with hematoxylin-eosin staining. Left: Large FOV with $\times 2.5$ magnification shows boundary between tumor and normal liver tissue (dashed line) and positions of A–F (open boxes A–F) at right in large FOV. Right: Images A–F at $\times 10$ magnification depict positions of iron-labeled microsphere (dark solid spheres) deposition. Microspheres accumulated primarily in isolated hepatic arteries and hepatic arterial branches of portal triads near tumor periphery. Those microspheres that were larger than selected $35\text{-}\mu\text{m}$ section thickness were crushed through microtome during slicing process, resulting in scratch artifacts (arrows, left) at these positions.

stantial shunting to the lungs and gastrointestinal tract should be excluded because of the risk of gastritis and pneumonitis (2). Alternatively, this may prompt the use of prophylactic measures (eg, proton pump inhibitors) in patients with identifiable extrahepatic deposition of microspheres. Our study establishes the feasibility of using MR imaging for detection of extrahepatic shunting at the time of infusion. An alternative preprocedural approach, similar to $^{99\text{m}}\text{Tc}$ -MAA studies, may be to inject nonradioactive tracer doses of iron-labeled microspheres to predict biodistribution prior to administration of a full dose of radioactive microspheres. The key benefit in preference to $^{99\text{m}}\text{Tc}$ -MAA would be that the tracer has exactly the same composition, physical characteristics, and, hence, biodistribution as the therapeutic radioactive dose.

Given the relatively poor spatial resolution and soft-tissue contrast of SPECT images with $^{99\text{m}}\text{Tc}$ -MAA, prediction of the intrahepatic biodistribution is gener-

ally not possible. Therefore, current ^{90}Y microsphere dosimetry calculations are based on the mass of the targeted lobar or segmental liver volume and do not account for differential microsphere uptake by the targeted lesion. Quantitative estimation of intrahepatic microsphere deposition was beyond the scope of our study. However, similar to promising ^{166}Ho studies, our initial results suggest the feasibility of future improvements to the current ^{90}Y microsphere dosimetry model by accounting for differential intrahepatic distribution within tumor and normal tissues. Although quantitative in vivo estimation of microsphere biodistribution may prove technically challenging, the clinical effect could be enormous, thus permitting dose optimization to maximize tumor kill while limiting toxic effects on normal liver tissues. Distribution-specific dose optimization may even permit the extension of this promising treatment modality to alternate organ systems.

The characteristic dipole image contrast patterns (18) demonstrated during

initial phantom studies were reproduced at in vivo positions of aggregate microsphere deposition when imaging in the coronal orientation parallel to the static magnetic field. For both NC and PC approaches, microsphere depiction was generally superior in the coronal orientation. Microsphere deposition generally occurred at the hypervascular periphery of the tumors. This was expected, given the necrotic central core of VX2 liver tumors.

Ambiguous signal voids and hyperintense regions within images with NC and PC can mimic those contrast patterns that result from iron-labeled microsphere deposition. Such ambiguities required direct observation of pre- and postinjection signal alterations for positive identification of microsphere deposition. These ambiguities can result from endogenous susceptibility differences between adjacent tissues, poor magnetic field shimming, and signals arising from abdominal fat tissues in images with PC. Fat signal was particularly problem-

atic for PC visualization of extrahepatic delivery. Fat saturation (not implemented for the current study) and differential depiction of these regions on images with NC should mitigate these ambiguities. However, for visualization of intrahepatic delivery, such ambiguities were less problematic because liver tissue signal was generally well suppressed in preinjection images with PC.

Significant CNR changes at positions of microsphere deposition were demonstrated for both NC and PC approaches. However, a rigorous comparison between these approaches was beyond the scope of this initial feasibility study. Signal intensity on NC and PC GRE images is strongly dependent on section thickness, echo time, and the section refocusing gradient moment. Likewise, the center frequency, bandwidth, and saturation pulse spectral profile each have a substantial effect on image contrast achieved with inversion-recovery with on-resonance water-suppression PC approaches (16). Further studies are necessary to optimize each approach and compare the sensitivity and specificity of each approach for in vivo detection of microsphere delivery.

There were limitations to our study. First, all images were obtained during free breathing. Superior image quality may likely be achieved with breath-hold or motion-synchronization techniques. Second, microsphere injections were performed by hand as opposed to using the pressure-limiting injection system typically used for microsphere infusion. In some cases, hand injections may have led to reflux of microspheres and unintended nontargeted delivery to adjacent liver segments as opposed to selective delivery to the tumor. Our study results should not be considered representative of ^{90}Y microsphere biodistribution after transcatheter delivery in liver cancer patients. Third, visualization of microsphere shunting to the lungs was not tested. MR visualization of iron-labeled microsphere deposition in the lungs could potentially be problematic due to susceptibility-induced static field gradients at air-tissue interfaces. Fourth, in vivo visualization was tested in a

small sample size of only seven animals. Fifth, labeling the microspheres with iron renders a paramagnetic property to the microspheres. Given the relatively small amount of iron contained in these microspheres, we do not anticipate significant redirection of these microspheres at clinical field strengths. However, further investigations are necessary to rigorously evaluate the effect of iron oxide labeling on overall biodistribution. Finally, in our study, we investigated only a single formulation of iron-labeled glass microspheres. Further studies with different formulations of iron-labeled microspheres are necessary to optimize iron oxide concentration, validate equivalent biodistribution to conventional nonlabeled microspheres, and evaluate the effect of iron labeling on ^{90}Y microsphere radiation dose. Blood flow dynamics in larger animals may differ from that in smaller animals, thus resulting in different microsphere distribution patterns. Additional preclinical studies in a larger animal model may be necessary for further methodological validation prior to clinical translation.

Practical application: Visualization of glass radioembolization microspheres by using conventional radiologic imaging modalities is challenging because of the limited spatial resolution of bremsstrahlung SPECT techniques (20).

References

1. Salem R, Thurston KG, Carr BI, Goin JE, Geschwind JF. Yttrium-90 microspheres: radiation therapy for unresectable liver cancer. *J Vasc Interv Radiol* 2002;13(9 pt 2):S223-S229.
2. Salem R, Lewandowski RJ, Atassi B, et al. Treatment of unresectable hepatocellular carcinoma with use of ^{90}Y microspheres (TheraSphere): safety, tumor response, and survival. *J Vasc Interv Radiol* 2005;16:1627-1639.
3. Salem R, Thurston KG. Radioembolization with yttrium-90 microspheres: a state-of-the-art brachytherapy treatment for primary and secondary liver malignancies. III. Comprehensive literature review and future direction. *J Vasc Interv Radiol* 2006;17:1571-1593.
4. Salem R, Thurston KG. Radioembolization with ^{90}Y microspheres: a state-of-the-art brachytherapy treatment for primary and secondary liver malignancies. II. Special topics. *J Vasc Interv Radiol* 2006;17:1425-1439.
5. Salem R, Thurston KG. Radioembolization with ^{90}Y microspheres: a state-of-the-art brachytherapy treatment for primary and secondary liver malignancies. I. Technical and methodological considerations. *J Vasc Interv Radiol* 2006;17:1251-1278.
6. Ackerman NB. Experimental studies on the circulation dynamics of intrahepatic tumor blood supply. *Cancer* 1972;29:435-439.
7. Nijssen JF, Seppenwoolde JH, Havenith T, Bos C, Bakker CJ, van het Schip AD. Liver tumors: MR imaging of radioactive holmium microspheres—phantom and rabbit study. *Radiology* 2004;231:491-499.
8. Seppenwoolde JH, Nijssen JF, Bartels LW, Zielhuis SW, van Het Schip AD, Bakker CJ. Internal radiation therapy of liver tumors: qualitative and quantitative magnetic resonance imaging of the biodistribution of holmium-loaded microspheres in animal models. *Magn Reson Med* 2005;53:76-84.
9. Bulte JW, Douglas T, Witwer B, et al. Magneto-dendrimers allow endosomal magnetic labeling and in vivo tracking of stem cells. *Nat Biotechnol* 2001;19:1141-1147.
10. Bulte JW, Duncan ID, Frank JA. In vivo magnetic resonance tracking of magnetically labeled cells after transplantation. *J Cereb Blood Flow Metab* 2002;22:899-907.
11. Geschwind JF, Artemov D, Abraham S, et al. Chemoembolization of liver tumor in a rabbit model: assessment of tumor cell death with diffusion-weighted MR imaging and histologic analysis. *J Vasc Interv Radiol* 2000;11:1245-1255.
12. Murthy R, Nunez R, Szklaruk J, et al. Yttrium-90 microsphere therapy for hepatic malignancy: devices, indications, technical considerations, and potential complications. *RadioGraphics* 2005;25(Spec Issue):S41-S55.
13. Yablonskiy DA, Haacke EM. An MRI method for measuring T2 in the presence of static and RF magnetic field inhomogeneities. *Magn Reson Med* 1997;37:872-876.
14. Smirnov P, Laverne E, Gazeau F, et al. In vivo cellular imaging of lymphocyte trafficking by MRI: a tumor model approach to cell-based anticancer therapy. *Magn Reson Med* 2006;56:498-508.
15. Seppenwoolde JH, Viergever MA, Bakker CJ. Passive tracking exploiting local signal conservation: the white marker phenomenon. *Magn Reson Med* 2003;50:784-790.
16. Stuber M, Gilson WD, Schär M, et al. Positive contrast visualization of iron oxide-labeled stem cells using inversion-recovery with ON-resonant water suppression (IRON). *Magn Reson Med* 2007;58(5):1072-1077.
17. Haacke EM, Brown RW, Thompson MR, Venkatesan R. *Magnetic resonance imaging: physical principles and sequence design*. New York, NY: Wiley-Liss, 1999.
18. Schenck JF. The role of magnetic susceptibility in magnetic resonance imaging: MRI magnetic compatibility of the first and second kinds. *Med Phys* 1996;23:815-850.
19. Kim HC, Chung JW, Lee W, Jae HJ, Park JH. Recognizing extrahepatic collateral vessels that supply hepatocellular carcinoma to avoid complications of transcatheter arterial chemoembolization. *RadioGraphics* 2005;25(Spec Issue):S25-S39.
20. Mansberg R, Sorensen N, Mansberg V, Van der Wall H. Yttrium 90 Bremsstrahlung SPECT/CT scan demonstrating areas of tracer/tumour uptake. *Eur J Nucl Med Mol Imaging* 2007;34:1887.