Magnetite Albumin Microspheres: A New MR Contrast Material

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A superparamagnetic MR contrast agent was synthesized by incorporating 150–250-Å particles of magnetite (Fe_3O_4, Fe_2O_3) in 1–5 μm human serum albumin microspheres. Magnetite albumin microspheres (MAM) target almost exclusively to the reticuloendothelial system after IV administration, are stable in vitro and in vivo, and possess a long shelf life. The agent has a large magnetic susceptibility effect that selectively reduces T2 with little effect on T1. Biodistribution studies that use a dose of 20 mg MAM/kg show prompt clearance from the blood pool with marked decrease in T2 for rat liver (40%) and spleen (45%) with a small decrease in liver (5%) and spleen (10%) T1 values. Pulmonary T1 and T2 decrease transiently over the first 24 hr, while no significant changes were observed in other tissues. Imaging of a rabbit VX2 tumor model resulted in a 200% increase in the contrast ratio of VX2 tumor to normal liver on T2-weighted and mixed T1-/T2-weighted pulse sequences after administration of contrast agent. The extreme potency, excellent targeting, and apparent lack of toxicity of this agent suggest that MAM probably will have a clinical application in detecting focal hepatic and splenic lesions.

Despite excellent soft-tissue contrast, MR imaging to date has shown no clear superiority over CT, sonography, or nuclear scintigraphy for the evaluation of focal liver disease [1]. Gadolinium DTPA (Gd-DTPA) contrast-enhanced MR studies of the brain have been shown to be helpful in characterizing CNS disease [2]. However, Gd-DTPA distributes randomly in the interstitial space, often obscuring rather than enhancing hepatic lesions by decreasing T1 differences of normal liver and tumor [2]. Chelates of paramagnetic ions shorten both T1 and T2, but their image contrast effect in the dose range studied is predominantly the result of reduction in T1. This study describes a new class of superparamagnetic contrast material that selectively enhances contrast between normal and pathologic tissue by augmenting T2 differences.

A reliable, safe reticuloendothelial-system (RES) MR contrast material that aids in differentiating normal and pathologic tissue has not been described. Tumor involvement of the liver consistently increases T1 and T2 relaxation but to a variable degree, resulting in false-negative MR studies if the chosen pulse sequences do not maximize contrast between tumor and normal tissue. Conditions resulting in abnormally high levels of parenchymal iron (such as transfusional hemosiderosis in liver, spleen, and pancreas or in the brain with chronic hemorrhage) result in decreased tissue signal on T1- and T2-weighted images [3–5], which suggests the potential use of particulate or aggregate nonsoluble iron compounds as T2 MR contrast material. This study evaluates subcapillary size magnetite albumin microspheres (MAM) as a liver/spleen MR contrast agent. MAM rapidly distributes to the RES and closely mimics the decrease in hepatic T2 seen in iron-storage disease states. The desired benefits of such an agent include (1) improved sensitivity in detecting and characterizing hepatic lesions, (2) shortening imaging time by using a predictable optimal pulse sequence, and (3) labeling RES function and turnover.

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Materials and Methods

Magnetite Albumin Microspheres: Preparation and Characterization

A modified water in oil emulsion polymerization method is used to prepare microspheres measuring approximately 1–5 μm in diameter and consisting of denatured human serum albumin matrix in which magnetite particles 150–250 Å in size are embedded (Fig. 1). In the experiments described, the following preparation was used [6]. An aqueous solution of human serum albumin (Sigma, St. Louis) and magnetite (Fe_3O_4) in the form of aqueous suspension (Ferrofluidics Corp., Nashua, NH) was made in distilled water. Aliquots of this suspension were homogenized in cottonseed oil (Sargent Welch, Skokie, IL) by sonication for 1 min. The homogenate was then added dropwise to stirred cottonseed oil kept at a constant temperature of 135°C. After 10 min, the emulsion was removed from the heat and stirred until cooled. Microspheres were washed free of the oil by centrifugation in anhydrous ether; they were washed free of ether and resuspended in 0.1% Tween 80 in 0.15 N saline. Microspheres were stored in a 10 mg/ml suspension. Before use, the microsphere suspension was vigorously agitated without sonication. The uniformity of size of the microspheres was checked with light microscopy.

Rat Biodistribution and Dose-Response Studies

Biodistribution and dose-response studies were obtained in male Sprague-Dawley rats weighing 300–400 g. The rats were anesthetized with intraperitoneal pentobarbital (35 mg/kg). Through a tail vein, injections were made of a serial concentration of 5–50 mg/kg MAM, 10% magnetite by weight. At 30 min, the animals were sacrificed by cervical dislocation, and tissues were obtained for T1 and T2 analysis. Samples included blood (obtained by cardiac puncture), liver, spleen, kidney, lung, and thigh muscle (obtained by excision). In all samples, T1 and T2 measurements were obtained within 45 min of death, and three samples were obtained at each dose and time point.

All T1 and T2 relaxation measurements were performed with an IBM PC-20 Minispec pulse MR spectrometer (IBM, Danbury, CT) at 0.47 T. Values of T1 and T2 represent an average of three consecutive measurements.

Normal Rat Imaging Studies

MR imaging of rats and rabbits was performed with a horizontal-bore (8 cm) superconducting magnet system (Technicare Corp., Solon, OH) at a magnetic field strength of 1.4 T. Images were acquired by using a two-dimensional Fourier transform technique with a 3-mm slice thickness and a 256 × 128 acquisition matrix. Various T1- and T2-weighted pulse sequences were used. Signal-to-noise (S/N) ratios were calculated as *tissue signal/background deviation*. S/N measurements were normalized for time (6 min/image) where S/N normalized = S/N ÷ $\sqrt{\text{acquisition time}}$.

Male Sprague-Dawley rats (approximately 400 g) were anesthetized, securely placed on a calibrated carrier, and inserted into the magnet. Tubes containing paramagnetically doped water or agar gels of known T1 and T2 were placed alongside the animal. Baseline images were obtained to optimize liver position within the imaging plane. After baseline images, animals were removed from the magnet, and IV MAM saline suspension was injected at a dose of 20 mg/kg animal weight. The position of the animal was not altered during the injection of MAM and the reinsertion of the animal into the magnet. Sequential imaging was performed at 2 and 4 months after infusion.

Rabbit VX2 Tumor Model

VX2, a transplantable carcinoma, was implanted by direct laparoscopic intrahepatic implantation into the liver of a New Zealand white rabbit weighing 800 g [7]. This tumor reaches approximately 1 cm in size and creates nodular metastases within the liver during the first 3 weeks after implantation. Serial images of the liver were obtained with various T1- and T2-weighted pulse sequences before and within 10 min after IV infusion of 20 mg MAM/kg into the rabbit ear. The change in contrast-to-noise (C/N) after MAM administration was calculated as preinfusion C/N − postinfusion C/N, determined by measuring C/N between normal tissue and tumor with T1, mixed T1/T2, and T2-weighted pulse sequences before and after MAM administration. C/N is defined as liver signal − tumor signal/background deviation [8].

Toxicity Studies.

Serial concentrations of MAM (100, 200, and 300 mg/kg) were injected in nine Sprague-Dawley rats (three rats/dose), and toxicity was assessed immediately and over the course of 1 week after injection.

Histologic Studies

Iron stain and collagen stains were obtained on liver histologic samples at 2 weeks and 2 months after MAM administration.

Results

The concentration of the agent in various tissues over time (biodistribution) is indicated by T1 and T2 changes caused by the agent.

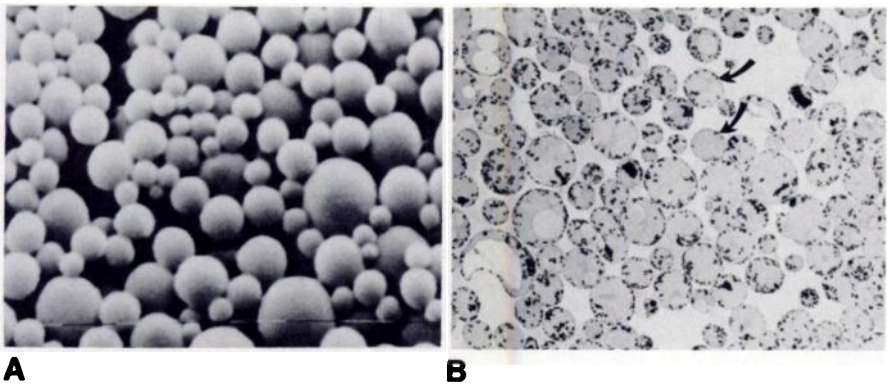

Fig. 1.—*A*, Scanning electron micrograph of MAM shows relative uniformity of spheres, which are subcapillary in size. Range of microsphere size used in this study is approximately 1–5 μm. Micrometer bar indicates size for both *A* and *B*.

***B*, Transmission electron micrograph of microspheres, which consist of shell of denatured human serum albumin in which 150- to 250-Å magnetite particles, dark spots (*arrows*), are embedded.**

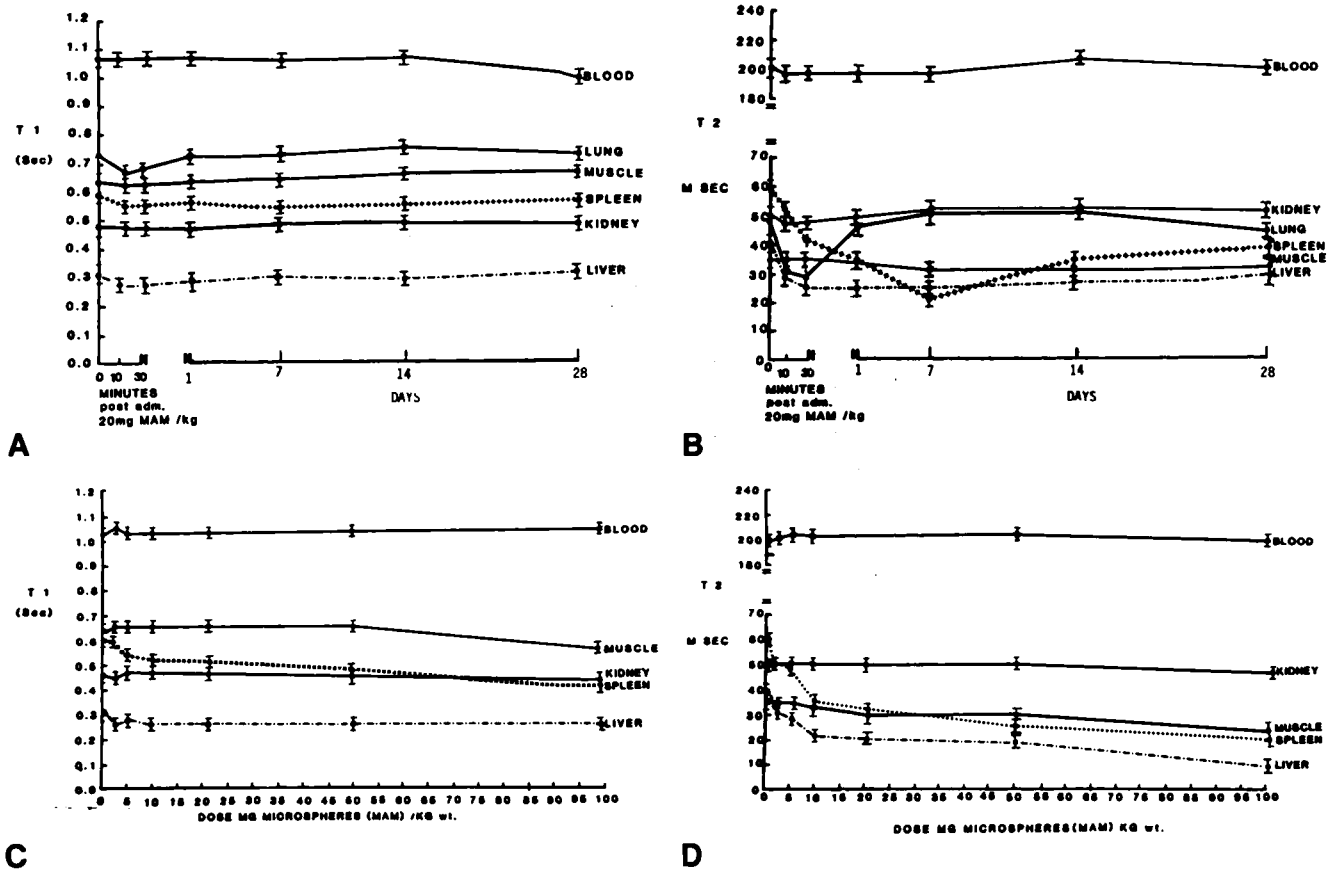

Fig. 2.—*A* and *B*, Longitudinal biodistribution. *C* and *D*, Dose/response data.

Results of biodistribution studies are summarized in Figure 2. The longitudinal biodistribution study (Figs. 2A and 2B) after administration of 20 mg MAM/kg shows no change in blood and renal T1 and T2 relaxation compared with precontrast control values, within 10 min after infusion. This indicates rapid clearance in the microspheres from the blood pool. There is a small decrease in hepatic and splenic T1 over 4 weeks. However, there is a dramatic 40% drop in hepatic and 45% drop in splenic T2 after microsphere administration. Hepatic T2 reduction persists for 4 weeks after infusion, but there is partial return to normal precontrast splenic T2 values, particularly at 2–4 weeks after infusion.

Dose-response data (Figs. 2C and 2D) show only a 10% decrease in splenic T1 and 5% decrease in hepatic T1 with increasing doses up to 100 mg/kg. There is a near-logarithmic decrease in hepatic and splenic T2 with increasing dose that is most pronounced in the low dose range. Blood, kidney, and muscle T1 and T2 are unchanged even with doses of 100 mg/kg.

Peak hepatic effect is observed within 10 min after infusion, with decrease in signal or blackening of the liver from decreased T2 in both strongly T1- and T2-weighted pulse sequences (Fig. 3). With all pulse sequences, hepatic signal decreases persistently at 2 and 4 months after infusion (Fig. 4). Liver histology specimens (n = 2) submitted for iron stain (obtained within 30 min after infusion of 20 mg MAM/kg) show exclusive uptake of microspheres by hepatic Kupffer cells (Fig. 5). Electron microscopy shows intracellular localization in phagosomes of resting monocytes or Kupffer cells (Fig. 6).

Within 20 min after a 20 mg/kg dose of microspheres in rabbits implanted with VX2 hepatic carcinoma, a marked reduction of signal occurs in normal liver. Tumor that replaces RES and has a long T2 appears bright because the dropout in signal of adjacent normal tissue and tumor margins is well defined (Fig. 7). The tumor appears as an area of increased signal with T2-weighted, mixed T1/T2 weighted, and even strongly T1-weighted pulse sequences. Tumor is most apparent on mixed T1/T2- and strongly T2-weighted pulse sequences with a 200% increase in the C/N after MAM infusion. Spectroscopy shows a 53% increase in T2 contrast ($T2_{tumor} - T2_{normal}$) between tumor and liver after MAM. The appearance of the tumor correlates well with gross pathology and histology of the liver tumor specimen.

A limited toxicity study with injection of 100, 200, and 300 mg MAM/kg (three rats/dose) in nine Sprague-Dawley rats (300-g males) resulted in no acute or subacute (1 week) deaths or apparent morbidity. At 2 months after administration of 20 mg MAM/kg, no hepatocellular damage or evidence of fibrosis was seen.

Discussion

Paramagnetic materials, which have one or more unpaired electrons, have been extensively evaluated as MR contrast agents. Enhancement results predominantly from T1 reduction. While iron in its ionic form is paramagnetic, clusters of iron ions create a collective domain, with a magnetic moment much greater than the sum of the paramagnetic ions. In this clustered or particulate form, iron is superparamagnetic or ferromagnetic. Particulate iron less than 300 Å in size, such

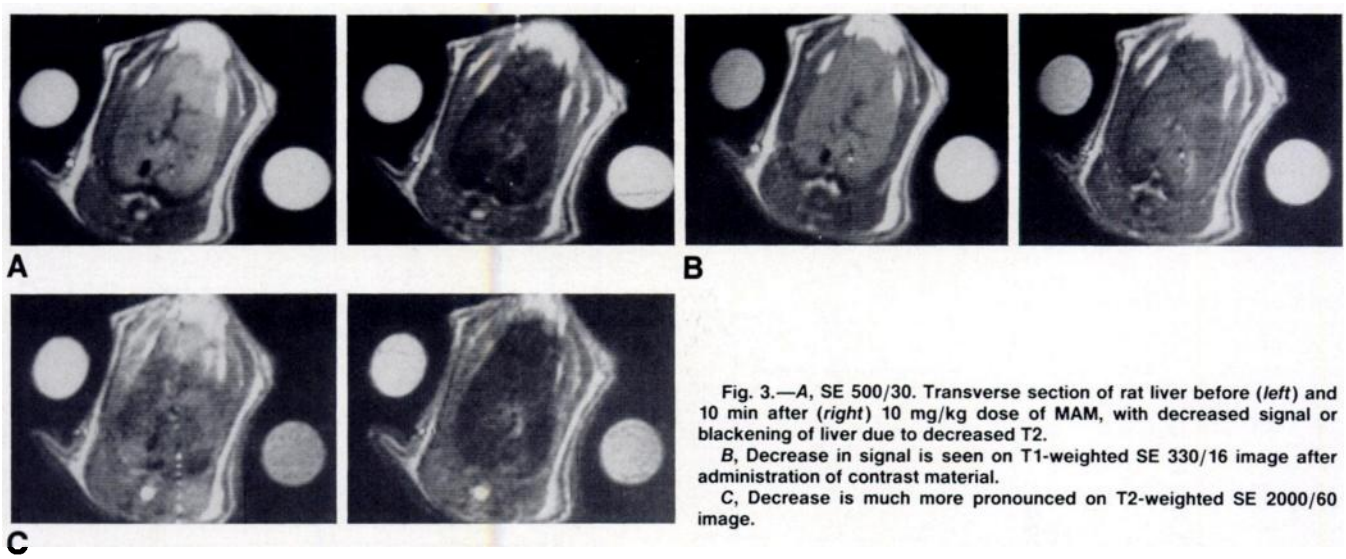

Fig. 3.—*A*, SE 500/30. Transverse section of rat liver before (*left*) and 10 min after (*right*) 10 mg/kg dose of MAM, with decreased signal or blackening of liver due to decreased T2.

B, Decrease in signal is seen on T1-weighted SE 330/16 image after administration of contrast material.

C, Decrease is much more pronounced on T2-weighted SE 2000/60 image.

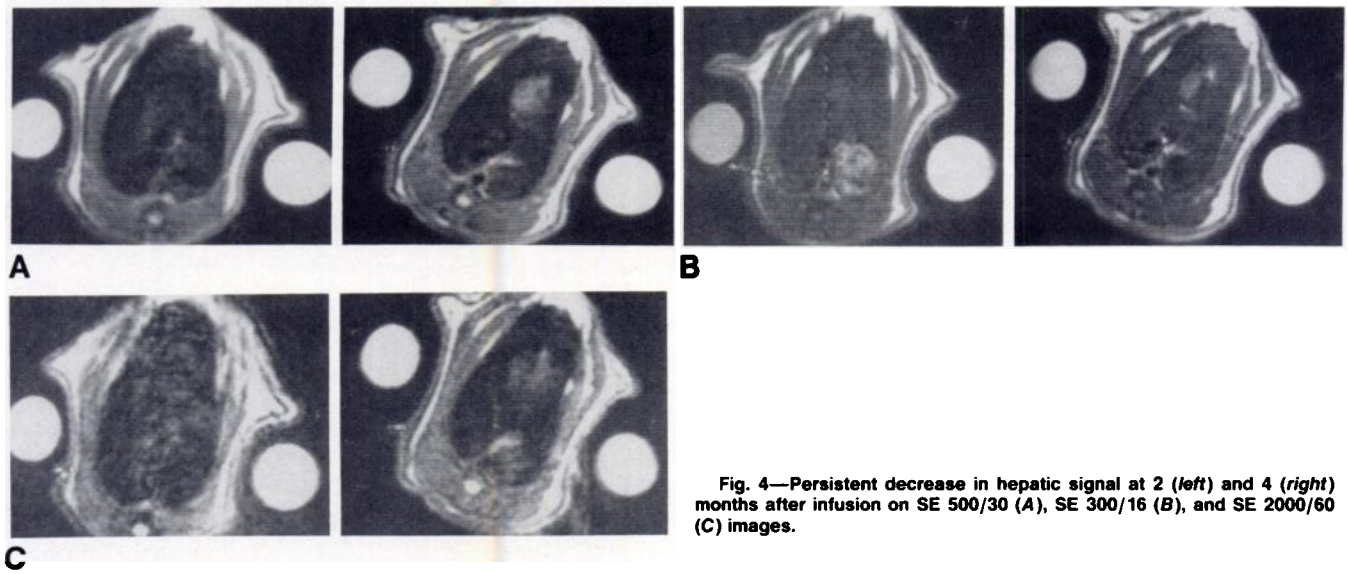

Fig. 4—Persistent decrease in hepatic signal at 2 (*left*) and 4 (*right*) months after infusion on SE 500/30 (*A*), SE 300/16 (*B*), and SE 2000/60 (*C*) images.

as magnetite, is superparamagnetic; larger particles are ferromagnetic. Both have a much larger magnetic susceptibility than paramagnetic material [9]. The use of ferromagnetic particles as an MR contrast material has been evaluated concurrently with this study [10].

Magnetization of MAM increases in a nonlinear manner with increased applied external field of 0.3–0.9 T, with minimal increase above 0.9 T [11]. Magnetization is rapidly lost when the superparamagnetic species is removed from the external field, unlike ferromagnetic materials, in which magnetization persists. This low remenance or residual magnetization prevents clumping or aggregation of microspheres because of attraction between magnetized particles that would adversely affect its biodistribution. The large magnetic moment of superparamagnetic material generates local field inhomogeneities and presumably promotes dephasing of proton spins and acceleration of transverse relaxation [12, 13]. Magnetite therefore exhibits a different relaxation mechanism than soluble paramagnetic contrast agents such as Gd-DTPA, which show equal enhancement of T1 and T2 relaxation, obeying the Solomon-Bloembergen equations [14]. The exact mechanism for promoting T2 relaxation is, however, not known and may be a combination of accelerated dephasing of proton spins from microfield inhomogeneity and local diffusion.

The marked enhancement of T2 relaxation with negligible T1 effects seen with magnetite is probably independent of

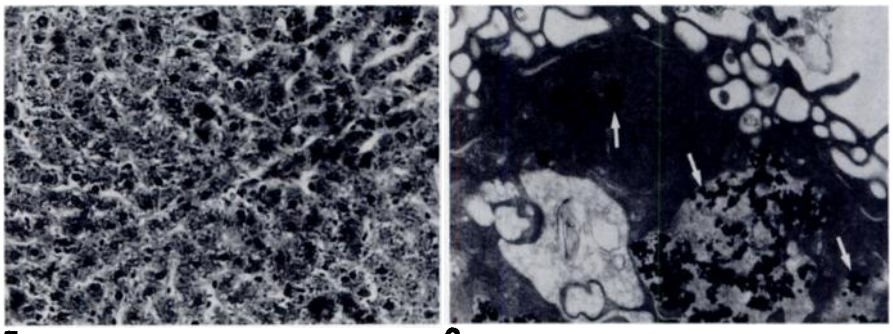

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Fig. 5—Liver histology specimen (iron stain) 30 min after infusion of 20 mg MAM/kg shows exclusive uptake of microspheres by hepatic Kupffer cells.

Fig. 6.—Intracellular localization of MAM in phagosomes of resting monocytes seen with electron microscopy (*arrows*).

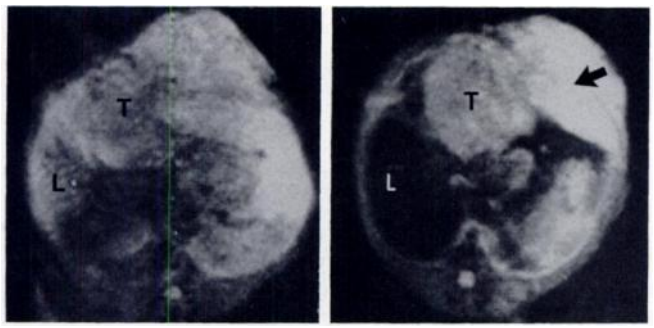

Fig. 7—Rabbit with implanted VX2 hepatic carcinoma. Transverse section of liver shows essentially isosignal mass (T) distorting hepatic architecture (L) on SE 500/30 pulse sequence. At 10 min postcontrast there is marked reduction of signal in normal liver. Tumor that replaces reticuloendothelial system has long T2, appears bright, and has well-defined margins. Extrahepatic component of tumor (*arrow*) is now apparent. Increase in tumor-liver contrast/noise is 200% after MAM.

direct contact with free mobile protons. Therefore, the coupling of the superparamagnetic species to a carrier should be achieved with minimal or no loss of magnetic susceptibility, unlike the adverse effects of chelation on paramagnetic species. The acquired magnetic moment depends on microsphere diameter, fraction of magnetite, and density, and it is expressed by the Langevin equation [10]. T2 effects may be observed with ferromagnetic materials. These materials are probably less desirable, however, because the long-term biologic effects of local remenant fields after magnetization in vivo are unknown.

Targeting of MAM is superior to that of previously described MR contrast materials. Water-soluble chelates such as Gd-DTPA distribute randomly in the vascular and interstitial space and can decrease T1 contrast between normal and pathologic hepatic lesions [2]. Our results confirm the rapid clearance of MAM by the RES, particularly hepatic, after IV injection. The RES, which has been the mainstay of radioscintigraphic study of liver and spleen disease, is the optimal target tissue for evaluating focal lesions, which are largely devoid of RES tissue. Hepatocytes appear protected from potential toxicity of MAM, which is sequestered in the RES, while they experience the local field inhomogeneities generated by the superparamagnetic agent. Significant tissue damage has not been seen even with iron-overload states where the RES is exclusively involved [3]. The long-term retention of MAM allows follow-up examination in patients being screened for metastatic disease without readministration of contrast material.

The main proposed use of MAM is for the improved detection of hepatic and splenic focal lesions that displace reticuloendothelial tissue. By shortening T2, MAM markedly reduces the signal from normal parenchyma, which contains phagocytic Kupffer cells. Because MAM can only enter target cells by phagocytosis [15], its localization is restricted primarily to intracellular phagosomes (Fig. 5). Uptake is excluded from most primary and metastatic neoplasms, since they are nonphagocytic. Our initial experience indicates that this agent should improve sensitivity in detection of focal liver lesions because of the markedly improved T2 contrast between tumor and normal parenchyma.

Previous toxicity studies in a large series of rats and mice have been encouraging; they show an LD_{50} of approximately 350 mg/kg (Widder KJ, unpublished data). Published toxicity studies in mice of MAM 1–1.5 μm in size reveal negligible adverse affects both in acute and chronic studies, even at 400 mg MAM/kg [16]. In our limited study in rats, no deaths or toxic effects were noted at doses up to 300 mg MAM/kg.

No immunogenicity has yet been noted in animal studies. No reports have indicated that microspheres or microaggregates promote intravascular coagulation, a theoretical problem with large albumin microspheres that are embolic in size, as well as with liposomes that provide a lipid surface for initiating the coagulation process.

We calculated a 20- to 250-fold margin of safety between the effective dose for imaging in rats and the extrapolated effective dose of MAM in humans vs the toxic dose seen in iron-overload states. The iron content of normal human liver has been reported as 0.15–0.20 mg iron/g liver wet weight. The iron content in symptomatic transfusional hemosiderosis is 1–10 mg iron/g liver wet weight [3]. The extrapolated effective dosage for a 70-kg adult, based on a dose of 15 mg MAM/kg (or 3 mg magnetite/kg) is 210 mg iron. With an average liver weight of 2200 g (range, approximately 1700–2800 g) and a 70% deposition in liver RES, a single dose of MAM would transiently increase total hepatic iron by 147 mg or 0.07 mg/g liver. The discrepancy in iron levels and T2 changes seen with MAM points out the potency of superpar-

amagnetic magnetite as compared with hemosiderin and other iron species seen in transfusional hemosiderosis. This reflects a much greater magnetic susceptibility of MAM as compared with hemosiderin (approximately 1000-fold) [17]. On histologic specimens obtained at 2 weeks and 2 months after MAM infusion, there was no evidence of hepatocellular uptake of iron, apparent hepatocellular damage, or hepatic fibrosis.

In conclusion, MAM is a new class of superparamagnetic MR contrast material that targets rapidly and predictably to the RES and exhibits superior targeting to paramagnetic chelates. This material strongly promotes T2 relaxation with minimal T1 effect, allowing for accentuation of T2 differences between normal and pathologic lesions (particularly tumors) with prolonged T2. It also allows for acquisition of T2-weighted images of the target organ by using shorter TR and TE pulse sequences. MAM are stable, relatively uniform in size, and appear nontoxic and nonallergenic in small animals. The iron levels delivered for effective imaging appear well below toxic levels seen in iron-overload states. We believe this type of agent offers considerable promise of improved and more efficient MR liver and spleen imaging.

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