Hepatocyte-Targeted MR Contrast Agents: Contrast Enhanced Detection of Liver Cancer in Diffusely Damaged Liver

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The performance of hepatocyte-targeted magnetic resonance (MR) contrast agents in the detection of liver tumor was tested in rats with hepatitis. Hepatocyte-targeted MR contrast agents (paramagnetic hepatobiliary complex [manganese-DPDP] and superparamagnetic iron oxide coated with arabinogalactan [SPIO-AG]) were injected into normal rats and rats with carbon tetrachloride-induced hepatitis. Before and after injection of either contrast agent, ex vivo relaxometry (0.94T) or in vivo MR imaging (1.0T) were performed. The obtained liver and tumor T1 and T2 relaxation times, liver and tumor signal-to-noise ratios (SNR), and tumor-liver contrast-to-noise ratios (CNR) of control rats and rats with hepatitis were compared.

Both relaxometry and MR imaging showed that MnDPDP and SPIO-AG selectively enhance liver tissue in controls and in rats with hepatitis to the same degree, and little tumor enhancement was seen in either group. As a result, no significant difference between control rats and rats with hepatitis was observed in the postcontrast tumor-liver CNR. For a MnDPDP-enhanced CNR with spin echo (SE) of 310/W, the results were −10.4 ± 3.6 in control rats vs. −11.5 ± 1.4 in rats with hepatitis; for a SPIO-AG-enhanced CNR with SE 2000/W45 and 2000/W90, respectively, the results were 30.7 ± 9.2 and 18.7 ± 4.7 in control rats vs. 31.9 ± 7.1 and 17.7 ± 2.4 in rats with hepatitis. These results indicate that hepatocyte-targeted contrast agents effectively enhance liver tissue and enhance liver-tumor image contrast despite hepatocellular dysfunction.

Keywords: hepatitis, liver neoplasms, contrast media, iron, manganese

Introduction

Diffuse liver diseases often mask liver cancer in ultrasound, CT (computed tomography), and MR (magnetic resonance) images1-5 because the echo level, density, and relaxation times of cancerous tissues overlap those of inflammatory processes.6,7 Hepatocyte-targeted pharmaceuticals can provide a mechanism for distinguishing cancer from the surrounding liver.8 Paramagnetic and superparamagnetic contrast agents developed for MR imaging have shown enhanced tumor-liver contrast in normal animals.9-11 Hepatitis was expected to reduce the uptake of hepatocyte-targeted contrast agents.12,13 If contrast agent uptake depends upon hepatocyte function, reduced uptake in areas of hepatitis would resemble tumorous tissue. Focal hepatitis would mimic cancer and diffuse hepatitis would mask cancer. However, if the uptake and enhancement of contrast agents is not affected by hepatocellular injury, these contrast agents could improve the detection of liver cancer despite the presence of hepatitis. To investigate the diagnostic efficacy of hepatocyte-targeted MR contrast agents, we have developed an animal model of liver cancer associated with hepatocellular injury.

Materials and Methods

Contrast agents

The hepatobiliary paramagnetic complex, manganese-dipyridoxal diphosphate (MnDPDP from Salutar Inc., now is available as Teslascan [mangafodipir trisodium] from GE Healthcare). The magnetic, biologic, and toxicological properties of this material have been reported in earlier studies, and the dosage of 15 µmol/kg has been...
chosen for experimental use.\textsuperscript{14-16} This material is selectively taken up via the cell membrane transport system in the liver and excreted into the biliary system.\textsuperscript{14,15} As tested in our laboratory, the longitudinal ($T_1$) and transverse relaxivities ($r_2$) of MnDPDP are 2.3 and 4.0 ($s^{-1} \cdot mM^{-1}$), respectively, in a 1% agar gel at 0.94T.

The superparamagnetic iron oxide particles (SPIO-AG) were obtained from Advanced Magnetics Inc., Cambridge, MA, USA. The particles are coated with arabinogalactan and targeted at the asialoglycoprotein receptor on the hepatocytes. The mean diameter of the particles is 12 nm.\textsuperscript{13,17,18} The magnetic, biologic, and toxicological properties and effective dosage (10 $\mu$mol/kg) of this material have already been reported.\textsuperscript{13,17,18} As tested in our laboratory, the $r_1$ and $r_2$ of SPIO-AG are 21 and 86 ($s^{-1} \cdot mM^{-1}$), respectively, in a 1% agar gel at 0.94T.

**Animal preparation**

Female Sprague-Dawley rats (Charles River Breeders, Wilmington, MA, USA) weighing 300–400 g were used in this study. Rat mammary carcinosarcoma (Walker 256, Biomeasure Inc., Hopkinton, MA, USA) was directly implanted into the liver according to previously described methods.\textsuperscript{19-21}

The rats were studied for 7–8 days after tumor implantation, when the tumor size reached a diameter of 0.5–1 cm. CCl\textsubscript{4} was selected as a well-characterized, reproducible, and widely available model of hepatocellular injury. Twenty four hours prior to study, 21 tumor-bearing animals were administered 0.4 ml/kg of a 1:1 mixture of carbon tetrachloride (CCl\textsubscript{4}) in corn oil via gastric intubation to induce chemical hepatitis.\textsuperscript{4,22} Tumor-bearing animals with or without hepatitis were studied before and after contrast administration. MnDPDP or SPIO-AG was injected via tail vein and the animals were studied 30 min or 60 min after contrast administration, respectively.

**Ex vivo relaxometry**

The longitudinal ($T_1$) and transverse ($T_2$) relaxation times were measured with a 0.94T MR spectrometer (PC-140 Minispec; Bruker, Milton, ON, Canada) at 40°C. $T_1$ was measured by means of a least-squares fit to eight data points generated with an inversion recovery pulse sequence. $T_2$ was measured from 10 data points generated by a Carr-Purcell-Meiboom-Gill pulse sequence. The $T_1$ and $T_2$ relaxation times of liver and tumor tissue were measured from 14 tumor-bearing animals with hepatitis and 12 tumor-bearing controls without hepatitis.

The enhancement attributable to the magneto-pharmaceuticals was expressed as the change in the $1/T_1$ and $1/T_2$ relaxation rates for a single tissue (liver or tumor). The pre- and post-contrast differences in the relaxation rates between tumor and surrounding liver were expressed as $\Delta 1/T_1$ and $\Delta 1/T_2 (\Delta 1/T_1,T_2 = 1/T_{1,2\text{before}} - 1/T_{1,2\text{after}})$. Statistical analysis was performed with the unpaired t-test.

**In vivo MR imaging**

Sixteen tumor-bearing rats (8 control animals and 8 animals with 24-hour-hepatitis, induced exactly as in the ex vivo studies) were employed for MR imaging (1.0T Magnetom; Siemens, Iselin, NJ, USA). $T_1$-weighted (SE 310/15, six excitations, 4.4-min scanning time) and $T_2$-weighted (SE 2000/45 and 2000/90, three excitations, 7.7-min scanning time) MR images were obtained before and after contrast injection. Four control rats (tumor only) and four rats with tumor and CCl\textsubscript{4}-induced hepatitis were employed for either the MnDPDP study or SPIO-AG study. The rats were placed supine in a head coil and axial multisection images were obtained with a section thickness of 3 mm (SE 310/15) or 4 mm (SE 2000/45 and 2000/90), a 1-mm interslice gap, and 256 x 128 matrix. The field of view was 16 cm in the frequency encoding direction and 11 cm in the phase encoding direction, such that the pixel size was 0.6 x 0.9 mm$^2$.

Signal intensities were measured by operator-defined regions of interest (greater than 50 pixels) of the tumor, liver, and background noise offset in the frequency encoding direction to avoid ghost artifacts.\textsuperscript{23} The signal-to-noise ratio (SNR) and the tumor-liver contrast-to-noise ratio (CNR) were calculated with standard methods (SNR = Signal/SDnoise; CNR = $S_{\text{tumor}} - S_{\text{liver}}$)/SDnoise).\textsuperscript{23-26}

For quantitative comparison of SNR and CNR data acquired with different pulse sequences, SNR and CNR values were normalized to scanning times and slice thickness with standard techniques.\textsuperscript{27,28} The significance of the differences between the SNR and CNR in controls and animals with hepatitis was evaluated with the unpaired t-test.

**Results**

**Ex vivo relaxometry**

In the absence of any contrast agent, liver $T_1$ and $T_2$ relaxation times were significantly ($P < 0.01$) increased after administration of CCl\textsubscript{4} (Table). The liver $T_1$ and $T_2$ showed a mean 16% increase (from 432 ± 17 to 499 ± 34 ms) and a mean 31% increase (from 40.9 ± 2.1 to 53.4 ± 4.4 ms). The tumor $T_1$ and $T_2$ relaxation times were not changed by CCl\textsubscript{4}-induced hepatitis. The difference between the tumor and liver $T_1$ relaxation rates ($\Delta 1/T_1$) was

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Table. *Ex Vivo* liver and tumor relaxation times (0.94T)

<table>
<thead>
<tr>
<th>Contrast Agent</th>
<th>Liver</th>
<th>Tumor</th>
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<tbody>
<tr>
<td></td>
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<td>T_1</td>
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<td>Control</td>
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<td>Hepatitis</td>
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<td>5</td>
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<tr>
<td>Control</td>
<td>MnDPDP</td>
<td>4</td>
</tr>
<tr>
<td>Hepatitis</td>
<td>MnDPDP</td>
<td>4</td>
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<tr>
<td>Control</td>
<td>SPIO-AG</td>
<td>4</td>
</tr>
<tr>
<td>Hepatitis</td>
<td>SPIO-AG</td>
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N: Number of animals. Controls: Tumor-bearing animals not injected with carbon tetrachloride. Hepatitis: Tumor-bearing animals with carbon tetrachloride-induced hepatitis. Each value shows a mean ± standard deviation of relaxation times (ms).

*, **The mean relaxation time is significantly greater (*: P < 0.01, **: P < 0.05) in the hepatitis groups than in the control groups.

^†The mean relaxation time is significantly decreased (P < 0.01) after the contrast injection.

significantly decreased (P < 0.05) in those with hepatitis (0.94 ± 0.22 s⁻¹) compared to the controls (1.21 ± 0.11 s⁻¹). The difference between the tumor and liver T_2 relaxation rates (∆1/T_{1,2}) was also significantly decreased (P < 0.01) in animals with hepatitis (8.1 ± 1.3 s⁻¹) compared to the controls (12.7 ± 1.5 s⁻¹). These results indicate that chemical hepatitis reduces the inherent relaxation rate difference (contrast) between the tumor and surrounding liver (Fig. 1).

MnDPDP: Injection of MnDPDP significantly decreased (P < 0.01) the liver T_1 and T_2 relaxation times in both controls and animals with hepatitis (Table). The liver T_1 in controls and in animals with hepatitis showed a mean 126% decrease (from 432 ± 17 to 191 ± 4 ms) and a mean 143% decrease (from 499 ± 34 to 205 ± 19 ms) with MnDPDP, respectively. MnDPDP increased the difference between the tumor and liver T_1 relaxation rate (∆1/T_1) by a mean of 134% in controls (from 1.21 ±

Fig. 1. *Ex vivo* tumor-liver contrast enhancement with contrast agents expressed as ∆1/T_{1,2} (Δ1/T_{1,2} = 1/T_{1,2liver} − 1/T_{1,2tumor})

In the unenhanced state, the differences in tumor-liver T_1 and T_2 relaxation rates (∆1/T_{1,2}) were significantly decreased (P < 0.05, P < 0.01) in the presence of hepatitis compared to the controls, indicating that hepatitis reduces the inherent tumor-liver relaxation rate difference (contrast). However, in the presence of hepatitis, MnDPDP or SPIO-AG increased the ∆1/T_1 or ∆1/T_2 to the same degree as in the controls, respectively. This result predicts that, in actual MR images, these contrast agents could sufficiently enhance the tumor-liver contrast even in the presence of hepatitis.
Fig. 2. Normalized liver SNR in SE 310/15, 2000/45, and 2000/90 images obtained before and after contrast injection
Left graph: MnDPDP study. Middle and right graph: SPIO-AG study.

A: The difference between normal controls and rats with hepatitis in terms of liver SNR in SE 310/15 images before and after MnDPDP injection was not significant (P > 0.05).

Middle and right graph: The difference between normal controls and rats with hepatitis in terms of liver SNR in unenhanced SE 2000/45 and 2000/90 images was significant (P < 0.05). After SPIO-AG injection, the difference was not significant (NS).

B: The difference between normal controls and rats with hepatitis in terms of tumor SNR was not significant (P > 0.05) before and after injection of either contrast agent.

0.11 s\(^{-1}\) to 2.83 ± 0.38 s\(^{-1}\); P < 0.01) and by a mean of 189% in animals with hepatitis (from 0.94 ± 0.22 s\(^{-1}\) to 2.72 ± 0.74 s\(^{-1}\); P < 0.01, Fig. 1).

SPIO-AG: Injection of SPIO-AG significantly (P < 0.01) decreased the liver T1 and T2 relaxation times in both controls and animals with hepatitis (Table). The liver T1 in controls and in animals with hepatitis showed a mean 37% decrease (from 40.9 ± 2.7 to 25.9 ± 3.0 ms) and a mean 54% decrease (from 53.4 ± 4.4 to 24.7 ± 2.5 ms) with SPIO-AG, respectively. SPIO-AG increased the difference between the tumor and liver T2 relaxation rate (ΔT1/T2) by a mean of 129% in controls (from 12.7 ± 1.5 s\(^{-1}\) to 29.1 ± 4.9 s\(^{-1}\); P < 0.01) and by a mean of 270% in animals with hepatitis (from 8.1 ± 1.3 s\(^{-1}\) to 30.0 ± 4.2 s\(^{-1}\); P < 0.01, Fig. 1).

In vivo MR imaging
The unenhanced liver SNRs and tumor SNRs in animals with hepatitis were not significantly different from those in the controls in the SE 310/15 images (Figs. 2A, B). Similarly, the tumor-liver CNRs in unenhanced T1-weighted SE 310/15 images showed no significant difference (P > 0.05) between control animals and animals with hepatitis (Figs. 3, 4). The unenhanced liver SNRs were significantly different from those in controls in
Fig. 3. Normalized tumor-liver CNR in SE 310/15, 2000/45, and 2000/90 images obtained before and after contrast injection.

Left graph: The difference between normal controls and rats with hepatitis in terms of tumor-liver CNR was not significant (P > 0.05) before and after MnDPDP injection.

Middle and right graph: The difference between normal controls and rats with hepatitis in terms of tumor-liver CNR was significant (P < 0.05) before SPIO-AG injection, but this difference diminished after SPIO-AG injection, indicating that tumor-liver contrast in the presence of hepatitis is unchanged with SPIO-AG injection. The CNRs are expressed as absolute values.

Fig. 4.

A: Unenhanced SE 310/15 images of a control rat (left) and a rat with CCl4-induced hepatitis (right). In this pulse sequence, the liver signal intensity in the rat with hepatitis (right) does not differ from that of the control rat (left). Hypointense tumors are seen in both animals (arrows).

B: SE 310/15 images of the control rat (left; same as A) and a rat with CCl4-induced hepatitis (right; same as A) 30 min after MnDPDP (15 μmol/kg) injection. The tumors (arrows) of both rats are more clearly demonstrated after contrast administration. The liver of the rat with hepatitis is well enhanced and there is no difference between the control rat (left) and the rat with hepatitis (right) in terms of tumor-liver contrast.

MnDPDP: The liver SNRs in the SE 310/15 T2-weighted SE 2000/45 and 2000/90 images (Fig. 2A), but the tumor SNRs were unchanged. As a result, the tumor-liver CNRs in animals with hepatitis were significantly smaller (P < 0.05) than those in the controls in T2-weighted SE 2000/45 and 2000/90 images (Figs. 3, 5).

MnDPDP: The liver SNRs in the SE 310/15 images were significantly increased (P < 0.05) in both controls and animals with hepatitis 30 min after MnDPDP injection (Fig. 2A). Tumor enhancement with MnDPDP slightly increased the tumor SNRs, but to a lesser degree than the increase in the liver SNRs (Fig. 2B). The tumor-liver CNRs in the SE 310/15 images were significantly increased (P < 0.05) by MnDPDP injection in the controls from −3.1 ± 0.2 to −10.4 ± 3.6 (235%) and increased from −4.1 ± 0.7 to −11.5 ± 1.4 (180%) in animals with hepatitis. The MnDPDP-enhanced tumor-liver CNRs in controls (−10.4 ± 3.6) and in
animals with hepatitis ($-11.5\pm1.4$) were not significantly different (Figs. 3, 4).

**SPIO-AG:** The liver SNRs in SE 2000/45 and 2000/90 images were significantly decreased (P $< 0.05$) in both controls and animals with hepatitis one hour after SPIO-AG injection (Fig. 2A). The tumor SNRs were unchanged (P $> 0.05$; Fig. 2B). The CNRs in the SE 2000/45 images were significantly increased (P $< 0.05$) by SPIO-AG injection in the controls, from 15.0 $\pm$ 5.6 to 30.7 $\pm$ 9.2 (105%), and in animals with hepatitis, from 7.5 $\pm$ 2.8 to 31.9 $\pm$ 7.1 (325%, Fig. 3). The CNRs in the SE 2000/90 images were also significantly increased (P $< 0.05$) by SPIO-AG injection in the controls, from 12.9 $\pm$ 4.0 to 18.7 $\pm$ 4.7 (45%), and in animals with hepatitis, from 7.6 $\pm$ 1.1 to 17.7 $\pm$ 2.4 (133%). The SPIO-AG-enhanced tumor-liver CNRs in T2-weighted images (SE 2000/45 and 90) in the controls (30.7 $\pm$ 9.2 and 18.7 $\pm$ 4.7) and in animals with hepatitis (31.9 $\pm$ 7.1 and 17.7 $\pm$ 2.4) were not significantly different (Figs. 3, 5).

**Discussion**

Our experimental model of liver cancer in the presence of chemical hepatitis resembles liver cancer occurring in alcoholics and in those with viral hepatitis and is therefore well suited for contrast media research in MR imaging. The model is simple, easy reproducible, and inexpensive and contains no risk of potential viral contamination.

Our results show that acute hepatitis does not prevent liver-specific relaxation enhancement by hepatocyte-targeted magnetopharmaceuticals. This finding contradicts published expectations that the uptake of hepatocyte-targeted contrast agents would be decreased in the presence of hepatitis. It has been documented that injured livers show sustained liver enhancement with MnDPDP and distinguish normal liver from hepatitis by not enhancing liver and by detecting cancer (by enhancing liver).

Previous expectations that a contrast agent could be used to detect cancer (by enhancing liver) and distinguish normal liver from hepatitis are somewhat contradictory. This study confirms that MnDPDP and SPIO-AG maintain cancer detection despite the presence of hepatitis.

Possible explanations for the preservation of liver-specific enhancement in the presence of hepatocellular injury are as follows: First, the biodistribution of the drug might not be changed at all, because the uptake mechanisms are not affected by the hepatic injury; second, the normal uptake mechanisms are impaired in hepatic injury, but the liver might have an alternative mechanism for drug uptake; third, while the uptake might be reduced, relaxivity might be increased in the altered intracellular environment; finally, it should be augured that the echo time used in T1-weighted SE 310/15 images may be too long to differentiate between normal and injured livers.

The first explanation might account for the enhancement with MnDPDP in hepatitis. It is not fully understood whether the uptake mechanism of MnDPDP is energy-dependent and whether it is the same as that of pyridoxal 5′ phosphate (PLP) or that of free manganese ions. In humans, it has been shown that the plasma clearance of PLP is increased in acute hepatitis. Evidence also exists that energy-independent processes allow the uptake of a substantial amount of Mn$^{2+}$ in the injured liver. The second explanation of an alternative uptake mechanism might account for the unchanged enhancement by SPIO-AG in hepatitis. It is known that galactose-bearing particles are taken up by hepatocytes via receptor-mediated...
endocytosis, an energy-dependent process that could be impaired in hepatic injury. If the uptake by hepatocytes were decreased, the unchanged liver uptake of SPIO-AG in hepatitis might be explained by the increased phagocytic activity of the Kupffer cells. Kupffer cells are also thought to have asialoglycoprotein receptor activity.34 For the third explanation, decreased but diffuse biodistribution of phagocytosed SPIO in the injured liver would play an important role in sustaining contrast enhancement.35,36 Finally, pulse sequences with shorter echo times might produce different results, and this explanation could be applied only to in vivo MR imaging data. Further studies will be needed to investigate the mechanisms of uptake and the enhancement of hepatocyte-targeted MR contrast agents.

Conclusion

The two hepatocyte-targeted contrast agents, MnDPDP and SPIO-AG, both increased tumor-liver CNRs in the presence of hepatitis to the same degree as in the controls.

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References