**MAJOR PAPER**

**Evaluation of Superparamagnetic Iron Oxide for MR Imaging of Liver Injury: Proton Relaxation Mechanisms and Optimal MR Imaging Parameters**

Akihiro TANIMOTO1*, Makio MUKAI2, and Sachio KURIBAYASHI1

Departments of 1Diagnostic Radiology and 2Pathology, School of Medicine, Keio University
35 Shinanomachi, Shinjuku-ku, Tokyo 160-8582, Japan
(Received May 2, 2006; Accepted July 5, 2006)

Purpose: To investigate the proton relaxation mechanisms and the optimal MR imaging parameters in superparamagnetic iron oxide (SPIO)-enhanced MR imaging of liver injury.

Methods: A liver injury model was created in the rat using carbon tetrachloride. The T1 and T2 relaxation effects of SPIO in normal and injured liver were estimated by ex vivo relaxometry. In vivo laser confocal microscopy of the liver was performed to simulate the distribution and clustering of SPIO particles in the hepatic macrophages. SPIO-enhanced MR imaging (1.5T) of normal and diseased rats was performed with variable parameters. The liver specimens were prepared for histopathological examination.

Results: Histopathological and laser confocal microscopic findings showed diffuse macrophage distribution but decreased intracellular clustering of SPIO in injured liver. Ex vivo relaxometry showed sustained T1 and T2 relaxation effects of SPIO in liver injury. On MR images obtained with moderate echo time (spin echo [SE] 2000/40 and gradient echo [GRE] 130/9.0/60°), injured liver showed significantly lower decrease in signal-to-noise ratio (SNR) than the normal liver, whereas little difference in SNR was found between the normal and injured liver on heavily T2-(SE 2000/80) and T1-weighted (SE 300/11 and GRE 130/2.0/90°) MR images.

Conclusion: Pulse sequences with a moderately long echo time (TE) may be more appropriate than heavily T1- or T2-weighted images for distinguishing normal and injured liver in SPIO-enhanced MR imaging because of the maintained T1 and T2 relaxation effect but decreased T2* relaxation effect of SPIO in injured liver.

Keywords: iron, contrast media, magnetic resonance, liver, hepatitis

**Introduction**

Superparamagnetic iron oxide (SPIO) particles have been applied in the detection by magnetic resonance imaging of focal hepatocellular lesions in chronic liver damage.1,2 The decrease in suppression of MR signal caused by SPIO in cirrhotic livers has been documented.3 A recent study showed SPIO-enhanced moderately T2-weighted and T2*-weighted GRE sequences to be the most sensitive sequences for detecting non-tumor hyperintensities indicative of liver fibrosis.4 However, few studies have been devoted to optimization of MR pulse sequence parameters to evaluate damaged livers. The proton T2 and T2* relaxation mechanisms by SPIO are influenced by many factors, such as particle size, magnetic properties, echo time, spatial distribution, and clustering of SPIO particles in tissues.5–10 Sparsely distributed SPIO particles show a decreased T2 relaxation effect.9 Recently, it has been reported that highly clustered SPIO particles exhibit a strong T2* (susceptibility) effect10 and that the T1 relaxation effect of SPIO is more pronounced in the cirrhotic liver as a result of the fine SPIO clustering.10,11 In this study, we determined the in vivo distribution of SPIO and the proton T1, T2, and T2* relaxation mechanisms in normal and injured liver and investigated the optimal MR imaging parameters for evaluating liver injury.

**Materials and Methods**

**Animal preparation**
The experimental protocols employed in this
study were approved by the Ethics Review Committee for Animal Experimentation of our institution. To alter the biodistribution of SPIO in the liver, we used a model of carbon tetrachloride (CCl₄)-induced hepatic injury. Hepatic injury was induced in male Sprague-Dawley rats (150–200 g body weight; Nippon Bio Supply Center, Tokyo, Japan) with 0.5 mL/kg of a 1:1 mixture of CCl₄ (Wako Pure Chemical Industries, Osaka, Japan) in corn oil, administered via a gastric tube.¹²

**Contrast agent**

We used ferumoxides (Feridex*: Eiken Chemical Co., Tokyo, Japan) as our SPIO particles. Ferumoxides is a 100- to 250-nm polycrystalline composite coated with dextran and is targeted to Kupffer cells (KCs) and other cells of the reticuloendothelial system (RES). The magnetic, biologic, and toxicologic properties of ferumoxides have already been studied in animals and humans using related iron oxide formulations.¹³

*Ex vivo* relaxometry

The longitudinal proton relaxation time (T₁) and transverse proton relaxation time (T₂) of rat liver specimens were measured with an MR spectrometer operating at 0.47T (NMS 120, Bruker Japan Inc., Tsukuba, Japan) at 40°C. T₁ was measured from 8 data points generated by an inversion recovery pulse sequence. T₂ was measured from 10 data points generated by a Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence, with tau values ranging from 0.3 to 1.0 ms, depending on the sample T₂ relaxation times, to observe 90% signal decay of the transverse magnetization for the calculation.

Liver specimens were obtained from 20 normal rats and 12 rats with hepatitis (24 hours after administration of CCl₄). Two groups, each comprising 10 normal rats and 6 rats with hepatitis, were further divided into subgroups that either received ferumoxides (10 μmol/kg body weight) or did not receive the contrast agent. Ferumoxides was administered via the tail vein 23 hours after the administration of CCl₄. The animals were sacrificed by decapitation 1 hour after the administration of ferumoxides, and liver samples were removed within 5 min of the sacrifice for *ex vivo* examination.

The E₁ and E₂ as the percentages of change in the T₁ and T₂ relaxation times, respectively, following the injection of SPIO were calculated for each animal as follows:

\[
E_1 (\%) = \frac{\text{Mean } T_1 \text{ (precontrast)} - T_1 \text{ (+ SPIO)}}{\text{Mean } T_1 \text{ (precontrast)}} \times 100
\]

The E₁ and E₂ were compared between normal animals and those with hepatitis by means of unpaired t-test.

**MR Imaging**

MR images were obtained on a 1.5T (Signa Horizon; GE Yokogawa Medical Systems, Hino, Japan) superconducting machine with T₁-weighted SE 300/11 (6 excitations); moderately and heavily T₂-weighted SE 2000/40 and 80 (2 excitations); heavily T₁-weighted fast-spoiled GRASS (FSPGR) 130/2.0/90° (2 excitations); and T₂*-weighted FSPGR 130/9.0/60° (2 excitations) sequences. The normal and diseased animals (6 rats each) were placed in pairs side by side in an extremity coil in the supine position, and axial multissection images were obtained with slice thickness of 4 mm, interslice gap of 1 mm, rectangular field of view of 14 cm×7 cm, and matrix of 256×256.

The signal-to-noise ratio (SNR) of the liver before and after injection of 10 μmol/kg of ferumoxides was obtained by dividing the signal intensity (S.I.) by the standard deviation (SD) of the background noise, which was determined outside the anterior-posterior phase ghost.¹⁴ The S.I. was determined by homogeneous region of interest (ROI) measurement of over 50 voxels, once each for the liver and the background noise. For the liver, the ROI box was usually placed in the right lobe as large as possible, excluding any major branches of the portal and hepatic veins. Because both signal and noise were measured on the same scale, no other normalization was conducted to compare the different pulse sequences.¹⁵

**Histology**

A pathologist scored the degree of iron accumulation in zone 1 (periportal zone) and zone 3 (perivenous zone) of hepatic lobules to confirm the heterogeneous distribution of SPIO in the CCl₄-induced liver injury model. Another group of rats (6 normal and seven with hepatitis) was administered 10 μmol Fe/kg of SPIO and sacrificed to obtain histological sections. Rat liver specimens were fixed in a 20% formalin solution for light microscopy using Berlin Blue iron staining. The degree of iron accumulation was scored as follows: 0 (no iron); 1 (scattered presence in a few KCs); 2 (scattered presence in several KCs); and 3 (abundant accumulation of iron particles).

**Laser confocal microscopy**

To measure the *in vivo* clustering of iron particles and their distribution in phagocytosing RES
cells, intravital laser confocal microscopy of the animal livers was performed. Hepatic injury was induced in Sprague-Dawley rats as described above. Four rats with hepatic injury and 2 normal rats as controls underwent laser confocal microscopy. The rats were anesthetized by intramuscular injection of pentobarbital sodium (50 mg/kg). The abdomen was exposed via a midline incision, and the left lobe of the liver was exteriorized using a previously described method. The surface of the liver was examined through an inverted-type intravital microscope assisted by line-scan laser confocal microscopy (Insight/TMD 300; Meridian Instruments Inc., Okemos, MI). The confocal fluorescence images were captured by an intensified CCD camera (C5810; Hamamatsu Photonics, Hamamatsu City, Japan) and displayed on a TV monitor (Trinitron HR, Sony, Tokyo).17

To simulate the in vivo functions of hepatic KCs and the SPIO biodistribution in these cells, 0.3 mL of 2.5%-solids carboxylate-modified and dyed latex beads (L4905; Sigma-Aldrich Japan, Tokyo) were injected intravenously. The mean size of the latex beads was 105 ± 4.7 nm (±SD), which was close to the particle size of ferumoxides (100- to 250-nm). The surface of the carboxylate-modified latex beads is negatively charged as a result of the presence of a hydroxyl group (−OH),18 and that of SPIO is also negatively charged because of the hydroxyl group of dextran.19 Because the selected size and surface structure of the latex beads were analogous to those of SPIO, it was expected that the phagocytosis of latex beads by RES cells would simulate that of SPIO.20 The fluorescent dye (yellow-green) covalently coupled to the surface of the latex beads was visualized at 505 nm by epi-illumination at 470 nm using an argon laser light source. Image acquisition was begun approximately 30 min after injection of the dye and continued for 120 min until the end of the experiments. Because of the structural heterogeneity of the liver,21,22 images were selected so that at least one hepatic lobule was included in the analysis.

The distribution and intensity of fluorescence of the accumulated latex beads were quantitatively assessed. Three full-color (16 million-color) images for each animal (12 from diseased and six from normal animals, 18 images total) were captured from different parts of the liver. All images were obtained under the same conditions of the laser confocal imager and amplifier gain of laser emission. The images were transferred from the target imaging camera to the computer (Power Macintosh G4; Apple Computer Japan, Tokyo), and then converted to binary (black and white) images for quantitative comparison. Image conversion was performed using the public domain NIH Image software (version 1.61, http://rsb.info.nih.gov/nih-image), setting the threshold at the 128th shade of the 256 gray scale, so that the fluorescent clustering of latex beads on the color images matched well with the clustering on the binary images by visual inspection.10 For each of the 18 binary images of the liver obtained, the clustered latex beads in the KCs were counted, and the area of each particle was calculated using the same software. The mean number of particles and the mean area of the particles were compared between the injured and normal liver by unpaired t-test.

**Results**

**Ex vivo relaxometry**

Both the T1 and T2 relaxation times were increased in the presence of liver injury. In terms of the proton T2 relaxation effect of SPIO, the mean E2 was 21.9% in the liver injury specimens, which was equivalent to a mean 22.4% in the normal liver (Table). The T1 relaxation effect of iron oxide (E1) was increased in the liver injury specimens as compared to that in the normal liver (P<0.05, significantly.

### Table. *Ex vivo* relaxometry of rat liver specimens at 0.47T

<table>
<thead>
<tr>
<th></th>
<th>Normal unenhanced</th>
<th>+ SPIO</th>
<th>Hepatitis unenhanced</th>
<th>+ SPIO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of rats</td>
<td>10</td>
<td>6</td>
<td>10</td>
<td>6</td>
</tr>
<tr>
<td>T1 (ms)</td>
<td>298 ± 14</td>
<td>277 ± 7</td>
<td>337 ± 10</td>
<td>298 ± 8</td>
</tr>
<tr>
<td>E1 (%)</td>
<td>6.9 ± 2.5%</td>
<td></td>
<td>11.6 ± 2.4%*</td>
<td></td>
</tr>
<tr>
<td>T2 (ms)</td>
<td>41.1 ± 2.5</td>
<td>31.9 ± 0.6</td>
<td>50.6 ± 1.2</td>
<td>39.6 ± 1.4</td>
</tr>
<tr>
<td>E2 (%)</td>
<td>22.4 ± 1.4%</td>
<td></td>
<td>21.9 ± 2.8%</td>
<td></td>
</tr>
</tbody>
</table>

*The difference between normal animals and animals with hepatitis is statistically significant (P<0.05). SPIO = superparamagnetic iron oxide.
Fig. 1. Liver SNR before and after administration of superparamagnetic iron oxide (SPIO)
a: Precontrast signal-to-noise ratio (SNR) on spin echo (SE) pulse sequences. Moderately T2-weighted (or proton density weighted [PDW]) and T2-weighted [T2W] SE images show increased SNR in animals with hepatitis.

b: Postcontrast SNR on SE pulse sequences. After administration of SPIO, a significant difference of SNR is noted on T1-weighted SE images between normal and injured liver.

c: Precontrast SNR on gradient echo (GRE) pulse sequences. No difference in SNR was found in either T1-weighted GRE or T2*-weighted GRE sequences between normal and injured liver.

d: Postcontrast SNR on GRE pulse sequences. After administration of SPIO, a significant difference in SNR was noted on T2*-weighted GRE sequences.

Table). Thus, acute hepatic injury caused by CCl4 did not aggravate the relaxation effect of SPIO.

MR Imaging
In the SE pulse sequences, liver SNR was significantly increased (P<0.01) on moderately T2-weighted SE 2000/40 (from a mean 48.6 to 58.7; Fig. 1a, Fig. 2a) and heavily T2-weighted SE 2000/80 (from 12.4 to 20.7; Fig. 1a, Fig. 2b) images in the animals with liver injury, whereas no significant difference was found on T1-weighted SE 300/11 sequences (from 152.9 to 151.2; Fig. 1a, Fig. 2c). After administration of SPIO, there was a significant difference in SNR on T1-weighted SE sequences between normal animals and animals with liver injury (88.2 and 155.3, P<0.01; Fig. 1b, Fig. 2c). A statistically significant difference of SNR was also noted on moderately (8.6 and 26.7, P<0.01; Fig. 1b, Fig. 2a) and heavily T2-weighted SE sequences (3.4 and 7.4, P<0.01; Fig. 1b, Fig. 2b).

In the case of GRE pulse sequences, there was no significant difference in SNR between normal animals and animals with hepatitis liver injury on either T1W-GRE (a mean 89.6 and 90.5; Fig. 1c, Fig. 2d) or T2*W-GRE sequences (119.0 and 116.0; Fig. 1c, Fig. 2e) before contrast enhancement. After administration of SPIO, on the other hand, there was a significant difference in SNR (P<0.01) on T2*W-GRE sequences between normal animals and animals with liver injury (46.3 and 67.7; Fig. 1d, Fig. 2e); however, no such difference was found on T1W-GRE sequences (101.0 and 93.1; Fig. 1d, Fig. 2d).
**Fig. 2.** Magnetic resonance (MR) imaging of a pair of normal and diseased animals (left; normal, right; diseased, upper column; precontrast, lower column; post administration of superparamagnetic iron oxide [SPIO])

**a:** On precontrast moderately T₂-weighted spin echo (SE) sequences, the injured liver (upper right) shows a slightly increased signal intensity compared with the normal liver (upper left). After administration of SPIO (lower column), the difference in signal intensity between normal (lower left) and injured liver (lower right) is more pronounced.

**b:** On precontrast heavily T₂-weighted SE, injured liver (upper right) shows apparently increased signal intensity compared with normal liver (upper left). After administration of SPIO (lower column), the difference in signal intensity between normal (lower left) and injured liver (lower right) is less distinct.

**c:** On precontrast T₁-weighted spin echo (SE) sequences, little difference in signal intensity is found between normal (upper left) and injured liver (upper right). After administration of SPIO (lower column), a difference in signal intensity is apparent between normal (lower left) and injured liver (lower right).

**d:** On both precontrast (upper column) and postcontrast (lower column) heavily T₁-weighted gradient echo (GRE) images, little difference in signal intensity is found between normal and injured liver.

**e:** On precontrast T₂*-weighted GRE, little difference in signal intensity is noted between normal (upper left) and injured liver (upper right). After administration of SPIO (lower column), a difference in signal intensity is apparent between normal (lower left) and injured liver (lower right).
Fig. 3. Histopathological iron-stained sections and distribution of macrophages with stainable iron (×200)

a: Normal liver shows KCs with stainable iron in a panlobular distribution.
b: Liver after administration of carbon tetrachloride (CCl₄) shows zone 3 (perivenous) necrosis and sparse distribution of stainable iron in intact zone 1 (periportal) and zone 2 (midzone).
c: Scoring of iron distribution shows sparse accumulation of iron in cases of liver injury. There is little stainable iron in damaged zone 3, resulting in a sparse biodistribution as compared with that in normal liver.

Fig. 4. A full-color laser confocal microscopic image of the liver (×40)

a: In normal liver, clustering of beads in KCs, which are hepatic macrophages residing in intrasinusoidal spaces, is visualized as brightly fluorescent yellow-green spots.
b: In injured liver, many, but only minute fluorescent yellow-green spots are noted.
Histology

Evaluation of the liver injury caused by CCl₄ revealed heterogeneous acinar damage, more pronounced in zone 3 (perivenous zone) than in zone 1 (periportal zone) hepatocytes (Fig. 3a, Fig. 3b). Scoring of the iron distribution in the livers revealed sparse accumulation of iron in cases of liver injury (Fig. 3c). There was little stainable iron in the damaged zone 3, explaining the sparse biodistribution in injured liver as compared to normal liver.

Intravital laser microscopy

Figure 4 illustrates a representative picture of hepatic microvascular units captured 30 min after the injection of latex beads (105 nm in size, equivalent to the size of ferumoxides). In normal liver, clustering of beads in KCs, which are hepatic macrophages residing in intrasinusoidal spaces, was visualized as brightly fluorescent yellow-green spots (Fig. 4a). By contrast, only minute fluorescent spots were noted in injured liver (Fig. 4b). Quantitative analysis showed that the mean area occupied by the latex beads (105 nm in size) was significantly smaller in injured liver (mean ± SD: 6.9 ± 9.4 × 10⁻⁵ mm²) than in normal liver (2.2 ± 2.7 × 10⁻⁴ mm², p < 0.001). On the other hand, the number of fluorescent particles in other RES cells in the injured liver (mean ± SD: N = 47 ± 25 per one image) did not differ significantly between injured and normal liver (N = 37 ± 45; difference not significant).

Discussion

Detection and quantitation of liver injury by medical imaging is challenging. There have been numerous attempts to assess liver function using radiolabeled colloids because it is generally accepted that most pathologic processes affect both KCs and hepatocytes. However, quantitation by scintigraphy fails to correlate with liver function because the particle delivery is affected by altered hepatic blood flow. Indirect manifestations, such as splenic accumulation of colloids, are not so reliable for diagnosis because they depend on blood flow and other secondary phenomena.

In practice, assessing phagocytic activity is one of the most important issues in relation to surgical intervention, especially liver transplantation and partial hepatectomy. Another practical consideration is estimation of the extent of liver damage after ablation or irradiation therapy. Several attempts have been made to quantitate hepatic injury by means of cell-specific magnetopharmaceuticals. However, the reduced enhancement in SPIO-enhanced MR imaging is not directly related to the degree of chronic liver damage, which limits the diagnostic value of this imaging technique in assessing chronic liver disease.

In our study, ex vivo relaxometry unexpectedly showed sustained T₁ and T₂ relaxation in injured livers, comparable to those in normal livers. In iron-stained sections, sparse distribution of stainable KCs was noted in injured livers, although some preserved uptake of iron was observed in uninjured acinar zones; therefore, saturable effects from cellular debris may not completely depress phagocytic function. Phagocytic function may be maintained by an influx of circulating macrophages in acute CCl₄ intoxication; however, the mechanism of uptake of SPIO in injured liver remains to be fully elucidated.

Decreased and sparse biodistribution of KCs in iron-stained sections indicates a decrease in the number of stainable phagocytosing KCs. In addition, laser confocal microscopic data revealed a maintained number of KCs, but decreased phagocytic activity of KCs in the injured liver tissue. In a previous study, we showed that the intracellular clustering of SPIO dominated the T₂* relaxation and that homogeneous distribution of clustered SPIO affected T₁ and T₂ relaxation. It may be possible that injured liver tissue contains a maintained number of KCs, but fewer intracellular SPIO clusters than normal liver parenchyma; this arrangement would allow water molecules to diffuse more readily into the vicinity of the SPIO clusters. Thus, the fine clustering of SPIO within newly implanted macrophages in the injured liver (which were not stainable on iron-stained sections but noted as faint latex accumulation on laser confocal microscopic images) may play an important role in the maintained T₁ and T₂ relaxation effect. It should be noted that the quantitative assessment of SPIO particle clustering by histochernistry is technically difficult because of the lack of linearity between the local staining intensities in tissue sections observed and the densities of the particles. Laser confocal intravital microscopy may be the method of choice to examine the spatial distribution of particles and their topographic relationship with RES cells because a paucity of heterogeneity in the fluorescence intensity among individual particles may allow quantitative evaluation of their clustering.

Pulse sequence optimization for evaluating hepatic injury has not been established. Mori and associates reported that the differences in signal intensity between livers with radiation injury and normal livers were better visualized on SPIO-
enhanced T1-weighted images than on SPIO-enhanced T2-weighted images, although SPIO-enhanced T2* -weighted fast field echo imaging was the most sensitive. We selected a relatively long TE (11 ms) for T1-weighted images. In our study, there was no significant difference in SNR between injured and normal liver on T2-weighted images obtained with a long TE; however, there was a marked difference in SNR between injured and normal liver on T1-weighted SE images obtained with a relatively long TE. Phase dispersion in proton T2 relaxation cannot be corrected by 180° refocusing pulse. On the other hand, phase dispersion caused by magnetic susceptibility/magnetic field inhomogeneity represents T2* relaxation and can be refocused by 180° pulse. Signal decrease from a strong T2 relaxation effect could be observed on T1-weighted SE images in the normal liver because of the relatively long TE. Conversely, hepatic injury may alter the spatial distribution and clustering of SPIO in vivo because of Kupffer cell dysfunction. Thus, T1 and T2 relaxation effect was maintained as exhibited in ex vivo relaxometry, but T2* relaxation effect of SPIO was decreased from the fine clustering of this magnetopharmaceutical in injured liver (although relaxometry cannot directly assess T2* relaxation effect). However, it remains uncertain whether decreased T2* relaxation effect causes decreased signal loss in injured liver on T1-weighted SE images with relatively long TE in that T1-weighted SE sequence is basically insensitive to T2* effect by 180° pulse. Similarly, it is speculative whether the T2* effect may be suppressed in normal liver on heavily T1-weighted GRE with very short TE, although little difference was found between injured and normal liver on this sequence.

The relatively maintained SPIO enhancement on heavily T2-weighted images in the injured liver could be explained by the high sensitivity of the pulse sequence to the drug and the maintained T2 relaxation effect. In fact, ex vivo T2 relaxometry using a heavily T2-weighted CPMG pulse sequence showed a maintained T2 relaxation effect in the injured liver. Therefore, pulse sequences with a moderately long or "halfway" TE would be more useful to distinguish injured normal livers than sequences with a very long (heavily T2-weighted SE) or very short TE. Modulated by the choice of the imaging pulse sequence, this effect can sometimes be expressed as increased signal intensity of injured liver.

The first priority in SPIO-enhanced MR imaging is undoubtedly the detection of focal hepatic lesions. Yamamoto's group reported that SPIO-enhanced MR imaging with moderately T2-weighted or FLASH sequences was highly accurate for detecting small hepatocellular carcinomas in cirrhotic livers. Our study revealed that pulse sequences with "halfway TEs" were more sensitive in detecting damage of the liver parenchyma than heavily T1- or T2-weighted sequences. It may be argued that pulse sequences sensitive to liver parenchymal change and those sensitive to focal hepatic lesions are opposite sides of the same coin. Nontumorous hyperintensities may mimic true focal hepatic lesions. Heavily T1- or T2-weighted pulse sequences would be required to eliminate the nontumorous hyperintensities when evaluating focal hepatic lesions.

There are some limitations to our study. First, we used chemically induced acute liver injury, which is not relevant to the clinical setting. Second, none of the quantitative data were correlated with the severity of liver damage. Third, magnetic field strength was different between ex vivo relaxometry and MR imaging. It is well known that magnetic field strength influences the relaxivity of SPIO. Further studies will be necessary to evaluate other hepatic injuries, such as those associated with cirrhosis, biliary obstruction, and radiation.

**Conclusion**

Hepatic injury may alter the spatial distribution and clustering of SPIO in vivo because of Kupffer cell dysfunction, resulting in maintained T1 and T2 relaxation effect but decreased T2* relaxation effect of SPIO. Thus, pulse sequences with a moderately long TE (proton density weighted SE and GRE with long TE) may be more appropriate than heavily T1- or T2-weighted images for distinguishing normal and injured livers in SPIO-enhanced MR imaging. This information could be applicable to future detection and quantitation of focal and/ or diffuse liver injury.

**Acknowledgements**

We thank Dr. Makoto Suematsu for his assistance with the laser confocal microscopy.

**References**


34. Lloyd RS, Triger DR. Studies on hepatic uptake of antigen. III. Studies of liver macrophage function in normal rats and following carbon tetrachloride administration. Immunology 1975; 29:253–263.