In vivo Evaluation of Exocytic Activity in Kupffer Cells using Superparamagnetic Iron Oxide-Enhanced Magnetic Resonance Imaging: an Experimental Study on Gadolinium Chloride-Induced Liver Injury in Rats.

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Introduction: Kupffer cells (KCs) are resident macrophages of the liver. Superparamagnetic iron oxide (SPIO) particles exhibit specific uptake by KCs, and SPIO-enhanced magnetic resonance (MR) imaging is useful for evaluating liver damage [1-3]. Although it is known that hepatic signal loss on SPIO-enhanced MR imaging is well correlated with phagocytic activity of KCs, few reports have focused on their exocytic activity to SPIO. Once compartmentalized within the lysosomes of KCs, iron oxide particles are broken down, with the majority of SPIO iron stored as ferritin and/or hemosiderin, which are antiferromagnetic forms of iron [4]. A recent study [5] indicated that when SPIO is administered before radiofrequency ablation of the liver, the signal decay of ablated liver parenchyma is prolonged because of impaired SPIO clearance. This suggests that delayed SPIO wash-out would be a useful marker for exocytic activity of KCs. We investigated the usefulness of SPIO-enhanced MR imaging to evaluate a series of functional activities from phagocytosis to exocytosis of KCs.

Materials and Methods: Male Wistar rats, weighting 230–250 g, were used for MR imaging. Rat livers were damaged by gadolinium chloride ($GdCl_3$) depending on the dose [6], which was injected intravenously. The dose of $GdCl_3$ was 7.5 mg/kg, 3.75 mg/kg, or 1.875 mg/kg, assigned to severe (n=6), moderate (n=3), and mild (n=3) injury groups, respectively. Control rats (n=3) were injected with the equivalent volume of normal saline. The next day, MR images were acquired before and after intravenous injection of SPIO at a concentration of 8 μmol Fe/kg, except for 3 rats in the severe injury group (severe injury without SPIO). Additional images were obtained 1 and 2 weeks after SPIO administration (8 and 15 days after administration of $GdCl_3$). All MR images were acquired with a 3 T whole body scanner (Signa HDx; GE, Milwaukee, WI) equipped with a dedicated receiver coil for small animal use. T_2*-weighted gradient-echo (TR/TE/FA = 450/11/30, 8 averages, 2 mm slice thickness, 80 mm FOV, 256 x 160 matrix) and T_2-weighted fast spin-echo (TR/TE = 4000/58, ETL = 8, 4 averages, 2 mm slice thickness, 80 mm FOV, 256 x 160 matrix) images were obtained. Signal intensities of the liver and paraspinal muscle were measured in the observer-defined region-of-interest. Relative intensity (RI) was calculated as the signal intensity of the liver divided by the signal intensity of the paraspinal muscle.

Results and Discussion: On T_2-weighted images, RIs before SPIO administration were 0.32 ± 0.09, 0.64 ± 0.06, 0.75 ± 0.10, and 0.79 ± 0.09 for severe, moderate, mild injury, and control groups, respectively (Fig. 1). On T_2*-weighted images, RIs before SPIO administration were 0.85 ± 0.04, 1.05 ± 0.03, 1.11 ± 0.04, and 1.16 ± 0.08 for severe, moderate, mild injury, and control groups, respectively (Fig. 2). The difference in RIs on T_2-weighted images between severe injury and control groups before SPIO administration was statistically significant ($p<0.05$, Tukey's test) and was possibly due to T_2 shortening by $GdCl_3$. On T_2-weighted images, mild and moderate injury groups showed almost the same reduction in RIs as the control group just after SPIO administration, suggesting that the uptake of SPIO was not much impaired by $GdCl_3$. On T_2*-weighted images, the reduction in RIs just after SPIO administration was small in the three hepatic injury groups, suggesting that the clustering of SPIO in KC lysosomes was impaired by $GdCl_3$. After 1 week, signal intensities of the liver on T_2*-weighted images were almost restored in the control group, and the differences in RIs between severe, moderate injury, and control groups were statistically significant (0.40 ± 0.04 vs. 1.00 ± 0.13, for severe injury vs. control, $p<0.01$; 0.58 ± 0.06 vs. 1.00 ± 0.13, for moderate injury vs. control, $p<0.05$). After 2 weeks, on T_2-weighted images, RIs were 0.20 ± 0.03, 0.30 ± 0.06, 0.53 ± 0.15, and 0.66 ± 0.06 for severe, moderate, mild injury, and control groups, respectively. On T_2*-weighted images, RIs were 0.42 ± 0.05, 0.68 ± 0.09, 0.84 ± 0.06, and 1.04 ± 0.10 for severe, moderate, mild injury, and control groups, respectively. Hepatic signals remained low in hepatic injury groups in a dose-dependent manner on T_2-weighted and T_2*-weighted images (Fig. 3a–d). The difference in RIs on T_2*-weighted images between severe injury and control groups was statistically significant ($p<0.01$, Tukey's test). In the severe injury group without SPIO administration, the signal intensity of the liver was almost stable during 2 weeks of follow-up (Fig. 3e). The delayed recovery of hepatic signal intensities 2 weeks later could be caused by damage to the exocytic activity of KCs to SPIO. The results suggest that evaluation of the exocytic activities of KCs is possible by serial MR imaging observations after a single dose of SPIO. This technique may be useful to evaluate hepatic injuries, even when signal loss just after SPIO administration does not reflect the severity of hepatic injury, for example, in mild and moderate injury groups in this model study.

Conclusion: SPIO-enhanced MR imaging would be useful to evaluate not only phagocytic but also exocytic activities of KCs.

References: [1] Radiology 1990; 174: 797-801. [2] Radiology 1992; 185: 163-168. [3] J Magn Reson Imaging 2008; 28: 1444-1450. [4] Cell Tissue Res 2004; 316: 315-323. [5] Radiology 2009; 251: 557-565. [6] J Leukoc Biol 1992; 52: 296-302.

Figure 1

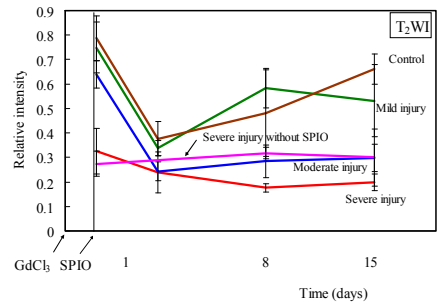
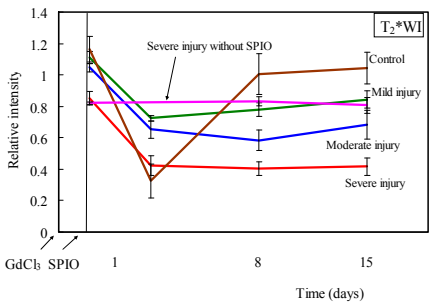


Figure 2



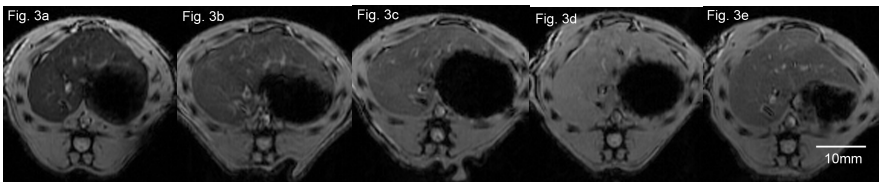


Figure 1 Relative signal intensities of the liver on T_2-weighted images. Data are the means ± SE.
Figure 2 Relative signal intensities of the liver on T_2*-weighted images. Data are the means ± SE.
Figure 3 Axial T_2*-weighted images obtained 2 weeks after the administration of SPIO. Hepatic signals are low in hepatic injury groups in a dose-dependent manner (a, 7.5mg/kg; b, 3.75mg/kg; c, 1.875mg/kg). Signal intensity of the liver is almost the same as the muscle in the control group (d). Hepatic signal of the severe injury group (7.5mg/kg) without SPIO administration is slightly low (e).

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