Introduction

Superparamagnetic iron oxide (SPIO) particles predominantly decrease T2* by magnetic susceptibility effect, and T2 by dipole-dipole interaction or scalar effect between nuclear spins and magnetic center [1]. Relaxation effects of contrast media are highly dependent on spatial distribution [2]. SPIO particles are phagocytosed and clustered intra-cellularly. Although gradient echo (GRE) pulse sequence is more susceptible than spin echo (SE) pulse sequences to magnetic field heterogeneity [3], a paradoxical phenomenon is observed in spleen enhancement: spleen shows a sufficient signal decrease by SPIO on T2-weighted fast SE, but not on GRE sequences. We investigated the effects of clustering on T2 (SE) and T2* (GRE) image contrast using in vivo and in-vitro models.

Methods

**MR Imaging for Phantoms**

We prepared three types of phantoms doped with SPIO (0.5 mM of ferumoxides; \( \varphi = 70-140 \) nm), consisting of only 1%-agar gel (A), both agar gel and Sephadex G25 superfine (F; \( \varphi = 40 \) nm), or G25 coarse (C; 350 \( \mu m \)) contained by 80% in volume [2]. The spatial distribution of ferumoxides in the Sephadex phantom is heterogeneous because it cannot get inside Sephadex.

MR images for phantoms were obtained at 1.5 T (Signa, GE) with T2-weighted SE (2000/40), heavily T1-weighted GRE (FSPGR 130/2/90'), moderately T1-weighted GRE (130/8.7/60'), and T2*-weighted GRE (FMPGR 130/7.7/30'). Signal intensity (S.I.) was obtained by homogeneous ROI measurements of each phantom.

**Intravital Laser Microscopy**

Wistar rats were used to observe the surface of the raw liver or spleen through an intravital laser confocal microscopy [4]. Because exogenous particles with the size ranging 80-215 nm are preferentially sequestered by Kupffer cells [5], the homogeneity of ferumoxides (\( \varphi = 70-140 \) nm) would be sequestered by the liver [6]. To simulate in vivo biodistribution of ferumoxides, fluorescent-dyed latex beads (\( \varphi = 105 \) nm) was injected intravenously. Another group of rats was injected fluorescent-dyed large latex beads (\( \varphi = 1 \mu m \)). The distribution and the strength of fluorescence from accumulated latex beads were quantitatively compared between the liver and spleen. Obtained full-color images were converted to binary (black & white) images, and clustered SPIO in Kupffer cells or macrophages were counted as particles. The number of particles and the mean area was calculated using a Macintosh software (NIH image) and was statistically compared between the liver and spleen by unpaired t-test.

**MR Imaging for Patients**

Fifty-eight consecutive patients were referred to ferumoxides-enhanced MR imaging due to suspicious of focal liver lesions. Before and approximately 30 to 120 minutes after the drip infusion of ferumoxides (10 \( \mu mol \) Fe/kg), patients underwent breathhold MR imaging consisting of T2-weighted fast SE [2700/80], moderately T1-weighted FSPGR (130/8.7/60'), and heavily T1-weighted FSPGR (140/2/90'). Signal-to-noise ratio (SNR) of the liver and spleen were obtained and the relative SNR decrease [\% SNRdecrease = \%SNRprecontrast - \%SNRpostcontrast / \%SNRprecontrast x 100 (%)] was calculated. To compare the effect of SPIO, %SNRdecrease was statistically analyzed between the liver and spleen by paired-t test.

Results

**MR Imaging for Phantoms** [Figure 1]

The spatial distribution of SPIO in the Sephadex phantom was heterogeneous with the order of the mean Sephadex size. The T2-weighted images for SPIO-doped phantoms showed that S.I. of the phantom with Sephadex [F, C] was higher than that of agar only [A]. On moderately T1-weighted FSPGR (130/8.7/60') and T2*-weighted FMPGR (130/7.7/30'; not shown), S.I. of [F, C] was lower than [A], in contrast to T2-weighted SE. On heavily T1-weighted FSPGR (130/2/90'), S.I. was higher in [F, C] than [A].

**Intravital Laser Microscopy** [Figure 2]

In the liver, the density of small latex beads (105 nm) was greater in periporal regions than in perivenous regions because of a relative paucity of Kupffer cells in the latter ones [5]. By contrast, only a small number of tiny spots were seen in the red pulp of the spleen. These results indicate that small latex beads (simulating ferumoxides) were predominantly uptaken in the liver macrophages to greater extents than in the spleen macrophages. On the other hand, the density of clustered large latex beads (1 \( \mu m \)) in Kupffer cells appeared almost equivalent to that observed in a red pulp region of the spleen in the same animal. Quantitative analysis showed that the number of clustered particles in RES cells was equivalent between the liver and spleen, but the mean of illuminating area was significantly smaller (p<0.0001) in the spleen (a mean 5.4x10^3 \( \mu m^2 \)) than in the liver (8.3x10^3 \( \mu m^2 \)) when small latex beads were injected. However, no significant difference was found (p=0.60) between the mean area of particles in the liver (3.1x10^3 \( \mu m^2 \)) and spleen (2.5x10^3 \( \mu m^2 \)) when large latex beads were injected.

**Discussion and Conclusion**

Both phantom study and intravital microscopy suggest that in vivo proton relaxation mechanism of SPIO is dependent on its clustering and spatial distribution. Agar-SPIO matrices surrounding Sephadex act as large particles like clustered SPIO in cells, causing a strong magnetic susceptibility (T2* effect). Spleen took up fewer particles than the liver, and the cluster size in splenic macrophages would be smaller than in hepatic Kupffer cells. The T2* effect depends on both the spatial distribution of SPIO and the cluster size, since larger cluster could produce gross local magnetic distortion [7]. Since T2 effect of SPIO is influenced by the interaction between water and magnetic center, the spatial distribution is more important than the cluster size. Even small particles can produce sufficient T2 relaxation effect on T2-weighted SE if they are homogeneously and densely distributed (in water solution or spleen). Clustering leads to the increase of the mean distance between the magnetic centers and water molecules, resulting in decreased T1 and T2 relaxivity. However, T1 effect in the spleen is less decreased than in the liver when clustering is small. As compared with the liver, T2* effect is decreased and T1 effect of SPIO is pronounced in the spleen. It follows that the signal increase of the spleen is much greater than the liver on heavily T1-weighted FSPGR.

In conclusion, enhancement of image contrast by SPIO is highly dependent on biodistribution, tissue sequestration, and the resultant spatial distribution of magnetic cores. Largely clustered SPIO particles in the liver produce greater signal decrease on GRE sequences than fine clustered SPIO in the spleen and blood.

**References**


**Figure 1**

**Figure 2 (x 40)**