Potential of Gd-EOB-DTPA for differential diagnosis of nonalcoholic steatohepatitis and fatty liver in rats using magnetic resonance imaging

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Synopsis

We investigated the potential of Gd-EOB-DTPA for the differential diagnosis of nonalcoholic steatohepatitis (NASH) and fatty liver (FL). NASH and FL rats were fed a choline-deficient diet or the standard diet containing 1 % orotic acid, respectively. After the feeding period, all rats were subjected to Gd-EOB-DTPA-enhanced MRI. Signal intensities were measured, and the relative enhancement (RE) was calculated. The time of maximum RE (Tmax) and elimination half-life of RE (T1/2) were compared. Tmax and T1/2 of NASH was significantly prolonged in comparison with FL. We could differentiate NASH and FL by evaluating the signal profile on Gd-EOB-DTPA-enhanced MRI.

Methods

Animal model

Twenty-one male Sprague-Dawley rats weighing about 150g were divided into three groups. Seven rats in the NASH group were fed a choline-deficient diet for 10 weeks. The seven rats in the FL group were fed the standard diet containing 1 w/w % orotic acid for 4 weeks. As a control, seven rats were fed the standard diet. During the entire study period, rats were maintained under special pathogen-free conditions at 22 ± 2 ºC with 12 hr artificial lighting from 7 am, and were fed chow pellets and water ad libitum.

Magnetic Resonance Imaging

After the feeding period, rats were subjected to contrast-enhanced MRI with 2D-FLASH(TR/TE=101/2.9 ms, FA 90º) using 1.0 T clinical imager under anesthesia with 40 mg/kg of pentobarbital sodium i.v. and 0.5 g/kg of urethane s.c. The field-of-view was 180 × 90 mm with a matrix of 256 × 128. The slice thickness was 5.5 mm, and the slice number was 12. The excitation number was 1, and the actual acquisition time was 6.4 sec. Gd-DTPA (0.1 mmol Gd/kg; Schering AG) and Gd-EOB-DTPA (0.025 mmol Gd/kg; Schering AG) were injected via the tail vein at intervals of 24 hrs. Nineteen images, including 3 precontrast measurements, were taken at intervals of 12 sec up to 3 min after injection of the contrast agent, which was injected immediately after acquisition of the third measurement. Subsequently, seven MR images were acquired at 5, 10, 15, 20, 30, 45, and 60 min after injection. The injection speed was about 1 mL/sec following 0.7 mL of saline flush.

Data analysis

Signal intensity (SI) of the liver was measured for each MR image, and RE was calculated with the following equation: RE(%)=[(SIpost-background)/(SIpre-background)]×100, where SIpre and SIpost are signal intensities of the liver before and after the injection of contrast agent. SIpre of the 2D-FLASH image was obtained from the third precontrast image in 19 continuous images. In addition, the time of maximum RE (Tmax) and elimination half-life of RE (T1/2) in the liver were compared.

Histological analysis

After MRI, the rats were sacrificed by deep anesthesia with sodium pentobarbital, and the livers were removed and subsequently fixed in phosphate-buffered 10 % formalin. The left lateral lobule of the liver was then divided into two sections at the long middle line, and the sections were embedded in paraffin blocks. Sections from each paraffin block were stained with hematoxylin and eosin (HE) or Sirius-red for histological examination. HE staining was used to observe liver pathologic structures, and Sirius-red staining was used to evaluate liver fibrosis.

Statistical Analysis

The difference in the Tmax and T1/2 of the liver after the injection of contrast agents in each group was examined by Tukey test (n = 7).

Results and Discussion

In histological findings of HE staining, diffuse macrovesicular steatosis and severe fibrosis were observed in the NASH group, whereas diffuse microvesicular steatosis and rare fibrosis were observed in the FL group. Lobular inflammation, which includes polynuclear leukocytes, and perisinusoidal fibrosis in zone 3 were also observed in the NASH group. According to the scoring system proposed by Brunt et al., NASH rats in this study were categorized with Grade 2 necroinflammatory activity and Stage 3 fibrosis. In histological findings of Sirius-red staining, perisinusoidal fibrosis in zone 3 and bridging fibrosis with nodular remodeling were observed in the NASH group, whereas there was rare fibrosis in FL and control groups. Therefore, comparative study using these animal models histopathologically may reflect the clinical situation to diagnose NASH and FL.

In the quantitative analysis of signal intensity, continuous increase and slow reduction of RE were induced after Gd-EOB-DTPA injection. However, RE in the liver of each group temporarily increased immediately after Gd-DTPA injection; thereafter, rapid reduction of RE was observed. In the comparison of Tmax after injection of the contrast agent, although there was no difference between Tmax in each group after Gd-DTPA injection, Tmax of the NASH group was significantly prolonged in comparison with FL and control groups after Gd-EOB-DTPA injection ($p<0.01$) (Table 1). Although Tmax in normal and FL groups was shorter than 3 min after Gd-EOB-DTPA injection, that in the NASH group was longer than 3 min (Figure 1). Although there was no difference between T1/2 in each group after Gd-DTPA injection, T1/2 in the NASH group was significantly prolonged in comparison with FL and control groups after Gd-EOB-DTPA injection, as for Tmax ($p<0.01$) (Table 2). Although T1/2 in normal and FL groups was shorter than 7 min after injection of Gd-EOB-DTPA, that in the NASH group was longer than 7 min (Figure 2).

In conclusion, this study showed the possibility of differentiating NASH from FL by comparing the signal profile after Gd-EOB-DTPA injection.

Table 1 Comparison of T_{max} in control, NASH, and FL group after injection of Gd-EOB-DTPA or Gd-DTPA (n=7)

	control	NASH	FL
Gd-EOB-DATA	2.5 ±0.2	5.1 ± 2.3**	2.6 ± 0.1
Gd-DTPA	0.2 ± 0.0	0.3 ± 0.1	0.2 ± 0.0

**: p<0.01, versus control and FL groups by Tukey test.

Table 2 Comparison of $T_{1/2}$ in control, NASH, and FL group after injection of Gd-EOB-DTPA or Gd-DTPA (n=7)

	control	NASH	FL
Gd-EOB-DATA	4.4 ± 1.4	8.9 ± 2.4**	4.2 ± 0.7
Gd-DTPA	1.0 ± 0.1	1.1 ± 0.2	0.9 ± 0.1

**: p<0.01, versus control and FL groups by Tukey test.

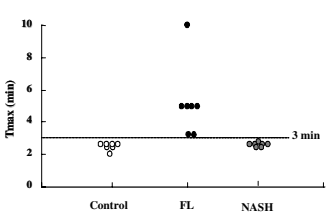

Figure 1 Comparison of Tmax in control, NASH, and FL groups after injection of Gd-EOB-DTPA (n=7).

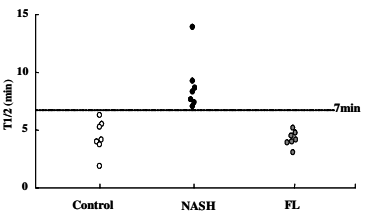

Figure 2 Comparison of T1/2 in control, NASH, and FL groups after injection of Gd-EOB-DTPA (n=7).