

New proposal for the staging of nonalcoholic steatohepatitis: Evaluation of liver fibrosis on Gd-EOB-DTPA-enhanced MRI

N. Tsuda¹, M. Okada², and T. Murakami²

¹Product Development, Bayer Yakuhin, Ltd., Osaka, Osaka, Japan, ²Department of Radiology, Kinki University School of Medicine, Osakasayama, Osaka, Japan

Introduction

The diagnosis of NASH is generally based on histological features, such as macrovesicular steatosis, lobular inflammation, and fibrosis observed in a biopsy. The extent of liver fibrosis is particularly used as an index of NASH staging, and the occurrence of perivenular, perisinusoidal, pericellular and/or bridging fibrosis, and liver cirrhosis are criteria for NASH staging. In a previous study, we reported that Gd-EOB-DTPA, which is a hepatobiliary contrast agent for MRI, would be useful to differentiate NASH from fatty liver in rats. In this study, we investigated the possibility of Gd-EOB-DTPA-enhanced MRI for NASH staging, using choline-deficient diet-induced NASH rats with poor, moderate or severe liver fibrosis.

Methods

Animal model

Twenty-eight male Sprague-Dawley rats weighing about 150g were split into four groups of seven. The rats in the NASH group were fed a choline-deficient diet for 4, 7, or 10 weeks. As a control, the remaining seven rats were fed a standard diet.

Magnetic Resonance Imaging

After the feeding period, the rats were subjected to contrast-enhanced MRI with 2D-FLASH; TR/TE=101/2.9 msec, nearly opposed-phase, flip angle 90° using a 1.0 T clinical imager (Magnetom, Harmony, GBSII, SIEMENS) under anesthesia. 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) and Gd-EOB-DTPA (0.025 mmol Gd/kg) were injected into the tail vein at intervals of 24 hrs. 19 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 via the tail vein.

Data analysis

The signal intensity (SI) of the liver was measured for each MR image, and relative enhancement (RE) was calculated with the following equation: $RE(\%) = [(SI_{\text{post-background}})/(SI_{\text{pre-background}})] \times 100$, where SI_{pre} and SI_{post} are signal intensities of the liver before and after injection of the contrast agent. In addition, the time of maximum RE (T_{max}) and elimination half-life of RE ($T_{1/2}$) in the liver were compared. The correlation between the fibrosis rate and T_{max} or $T_{1/2}$ was statistically evaluated.

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. A section from each paraffin block was stained with hematoxylin-eosin (HE) to observe the liver's pathologic structures or Sirius red to evaluate liver fibrosis. Computer-assisted fibrosis analysis was performed using the WinROOF image processing software which allowed to measure the area designated by the certain color ratio. The whole area and fibrosis stained with Sirius red were measured based on each threshold value of color ratio, and the fibrosis rate (%) was calculated with the following formula: fibrosis/whole area×100.

Statistical Analysis

The difference in T_{max} and $T_{1/2}$ of the liver after the injection of contrast agents in each group was evaluated with one-way analysis of variance (ANOVA) and Tukey post-hoc test ($n=7$). The Pearson product-moment correlation coefficient was used to evaluate the relationship between the liver fibrosis and the T_{max} or $T_{1/2}$.

Results and Discussion

In the histological findings from HE staining, diffuse macrovesicular steatosis (>66%) was observed in each treated group, whereas no steatosis was observed in the control group. In the results of Sirius red staining, various levels of fibrosis were induced in the treated groups (Fig.1). Perisinusoidal fibrosis in zone 3 was observed in the 7 and 10 week-treated groups, and bridging fibrosis with nodular remodeling were observed in the 10 week-treated group. On the other hand, there was rare fibrosis in the control and 4 week-treated groups. The fibrosis rate of the 10 week-treated group was significantly higher than the control and 4 or 7 week-treated groups ($p<0.01$) (Fig 2). In comparison of the entire signal, a continuous increase and slow reduction of RE in the further time course after peaking at T_{max} were induced after Gd-EOB-DTPA injection; however, RE in the liver of each group temporarily increased immediately after Gd-DTPA injection; thereafter, a rapid reduction of RE was observed. In the comparison of T_{max} after injection of the contrast agent, T_{max} of the 10 week-treated group was significantly prolonged in comparison with the control and 4 or 7 week-treated groups after Gd-EOB-DTPA injection ($p<0.01$), although there was no difference between T_{max} in each group after Gd-DTPA injection. Comparing the fibrosis rate and the T_{max} after Gd-EOB-DTPA injection, there was a significant correlation between both ($r = 0.90$, $p < 0.01$) (Fig 3). While there was no difference between the $T_{1/2}$ in each group after Gd-DTPA injection, the $T_{1/2}$ in the 10 week-treated group was significantly prolonged in comparison with the control and 4 or 7 week-treated groups after Gd-EOB-DTPA injection, and the same was true for T_{max} ($p<0.01$). Comparing the fibrosis rate and $T_{1/2}$ after Gd-EOB-DTPA injection, a significant correlation was found between both ($r = 0.97$, $p < 0.01$), as well as the T_{max} (Fig 4). In conclusion, this study clearly indicated that Gd-EOB-DTPA-enhanced MRI is useful to assess the progress of liver fibrosis in NASH. We believe that this invasive technique is a clinically feasible technique for diagnosis in NASH staging.

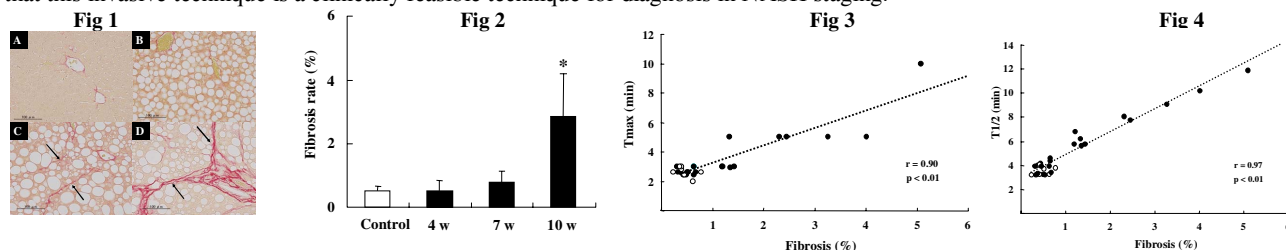


Fig.1 Typical histological sections of the control (A) and 4 (B), 7 (C), 10 (D) week-treated groups by Sirius red staining.

Fig.2 Comparison of the fibrosis rate in the control and 4, 7, or 10 week-treated groups. *: $p < 0.01$ versus the control and 4 or 7 week-treated groups.

Fig.3 Correlation between the T_{max} and fibrosis rate after injection of Gd-EOB-DTPA at 0.025 mmol Gd/kg.

Fig.4 Correlation between the $T_{1/2}$ and fibrosis rate after injection of Gd-EOB-DTPA at 0.025 mmol Gd/kg.