The Possibility of Differentiation between Nonalcoholic Steatohepatitis and Fatty Liver in Rabbits on Gd-EOB-DTPA-enhanced Open-type MRI Scans

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Rationale and Objectives: We used rabbits to investigate the possibility of differentiating between nonalcoholic steatohepatitis (NASH) and fatty liver (FL) on scans acquired by open-type– and gadolinium-ethoxybenzyl-diethylenetriamine pentaacetic acid (Gd-EOB-DTPA)–enhanced magnetic resonance imaging (MRI).

Materials and Methods: We divided 15 adult rabbits into three equal groups; they received standard (control group), high-fat (FL) content (FL group), or choline-deficient chow (NASH group). With the animals under general anesthesia we acquired scans on an open 0.3-Tesla MRI system. Signal intensity (SI) was measured before and after contrast administration and defined as SI-pre and SI-post, respectively. Relative SI enhancement (Sr) was calculated using the equation: Sr = (average of three SI-post- minus average of three SI values in no-signal fields)/(average of three SI-pre- minus average of three SI values in no-signal fields) × 100. Maximum Sr (Srmax), the time (in seconds) required to reach Srmax (Tmax), and the difference between Srmax and Sr at 30 minutes ($Sr_{30m}R$) were analyzed.

Results: Srmax was significantly higher in the NASH rabbits than the other two groups ($P < .05$).

Conclusions: In rabbits, the Srmax value made it possible to differentiate NASH from normal and fatty liver.

Key Words: Digestive system; MRI; animal research; Gd-EOB-DTPA.

Nonalcoholic steatohepatitis (NASH) was first named by Ludwig et al to describe biopsy findings in patients with no history of significant alcohol consumption (1). In autopsy studies performed in the United States, approximately 20% of liver specimens revealed hepatic steatosis and about 3% manifested NASH. NASH has also been reported in children (2,3). Approximately 1% of hepatic steatosis advances to NASH and 40% of NASH is considered fibrotic; 3%–10% of cases advance to liver cirrhosis (4–6). Therefore, early detection and treatment are important (7). At present, there are no biochemical diagnostic tests for NASH and biochemical studies may return normal results even in patients with serious liver disease. An unequivocal clinical diagnosis requires liver biopsy (2).

Criteria for the evaluation of hepatic magnetic resonance imaging (MRI) scans have not been established (4). In their rat experiments, Tsuda et al (8) used a 1.5 Tesla MRI scanner and gadolinium-ethoxybenzyl-diethylenetriamine pentaacetic acid (Gd-EOB-DTPA), a hepatobiliary contrast agent in rats, to differentiate between NASH and fatty liver (FL). Because different species and MRI scans acquired at different field strengths may affect the results, we subjected rabbits with NASH induced by a choline-deficient diet, rabbits with FL induced by a high-fat diet, and rabbits fed a standard diet to open-type MRI scanning.

MATERIAL AND METHODS

Our study protocol was approved by the Animal Experimentation Committee of our institute; all experiments were performed according to the Animal Care guidelines of our institution.

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Rabbit NASH Model

We randomly divided 15 adult female Japanese white rabbits (2.5–3.0 kg, SLC, Tokyo, Japan) into three equal groups. The NASH group (n = 5) was fed a choline-deficient diet for 10 weeks to induce NASH (basal diet F2PR, Oriental Yeast Co. Ltd, Tokyo, Japan). The FL group (n = 5) received a 1% cholesterol-containing diet for 10 weeks to induce FL (basal diet RC4, Oriental Yeast Co.). The controls (n = 5) were fed a standard rabbit diet for 10 weeks.

MRI

Scans were acquired on a horizontal open 0.3 Tesla MRI system (AIRIS II comfort, Hitachi Medical Corp, Tokyo, Japan); the scanning parameters were: knee coil (150 mm in diameter), field of view 150 mm, bandwidth 28.5 kHz, repetition time 170 ms, echo time 12.0 ms, flip angle 60°, slice thickness 5.0 mm, slice interval 6.0 mm, number of signals averaged 20, image matrix 256 × 256. Each rabbit was anesthetized with an intramuscular injection of medetomidine hydrochloride (0.1 mg/kg, Meiji Seika Co. Ltd, Tokyo, Japan) and ketamine hydrochloride (25 mg/kg, Sankyo Yell Yakuhin Co. Ltd, Tokyo, Japan) and fixed in the prone position in the coil. Gd-EOB-DTPA (0.025 mmol Gd/kg, Bayer Healthcare Co. Ltd, Tokyo, Japan) was manually injected into the auricular vein. In the course of 30 minutes we acquired three precontrast MRI scans; postcontrast scans were obtained at 45-second intervals. The room temperature was maintained at 25°C with an air conditioner.

Data Analysis

Hepatic signal intensity (SI) was assessed using Photoshop 7.0.1 (Adobe Systems Inc, San Jose, CA). After two radiologists consensually identified the first MRI slice on which the gallbladder appeared, signals from the right, middle, and left liver lobe in the same cross section were measured. For background we measured SI in the nonsignal field on the corresponding ventral side. A region of interest of 10 × 10 pixels was placed. SI before and after the administration of Gd-EOB-DTPA was defined as SI-pre and SI-post, respectively. The relative enhancement in SI (Sr) was calculated using the equation: Sr = (average of three SI-post- minus average of three SI values measured in no-signal fields)/(average of 3 SI-pre- minus average of three SI values measured in no-signal fields) × 100. We compared the maximum Sr (Srmax) values obtained in 30 minutes after contrast enhancement, the time (in seconds) to Srmax (Tmax), and the difference between Srmax- and Sr values recorded 30 minutes postcontrast enhancement ($Sr_{30m}R$) among the three groups of rabbits.

Statistical Analysis

Differences in Srmax, Tmax, and $Sr_{30m}R$ values were analyzed by one-way analysis of variance and Tukey's post-hoc test. Differences of $P < .05$ were considered statistically significant. For statistical analysis we used Dr. SPSS II for Windows (SPSS Japan Inc, Tokyo, Japan).

Histological Analysis

After scanning, the rabbits were sacrificed by a cardiac injection of an overdose of pentobarbital (Dainippon Sumitomo Pharma Co. Ltd, Tokyo, Japan). Their livers were harvested and maintained in 10% buffered formalin for 72 hours before paraffin embedding. One 4-μm thick slice was deparaffinized and stained with hematoxylin and eosin. In FL- and NASH rabbits, one pathologist, blinded to the group from which the specimen derived and to the experimental protocol used, evaluated the NASH score (9,10). The scores for nonalcoholic FL disease (NAFLD) were based on histological features; a score of 0–3 was assigned to steatosis, of 0–2 to lobular inflammation, and of 0–2 to hepatocellular ballooning. NASH was recorded when the sum total of the assigned scores exceeded 5. NASH was then staged and graded as follows. Stage 1 was recorded in the presence of fibrosis in zone 3, stage 2 if there was stage 1 periportal fibrosis, stage 3 if there was central-portal bridging fibrosis, and stage 4 when cirrhosis was identified. NASH grading was based on the constellation of lesions considered important in steatohepatitis (ie, steatosis, ballooning, and lobular and portal inflammatory infiltrates).

RESULTS

MRI

Typical images obtained before, and 90 seconds, and 4 and 30 minutes after contrast administration in the NASH, FL, and control group are shown in Figure 1. In all groups there was an immediate increase in SI after contrast injection. However, there was no change in SI at 30 minutes postcontrast administration.

Sr of the Liver Parenchyma

In all three groups, the signal from the liver parenchyma peaked within 5 minutes after contrast administration (Fig 2). Tmax, Sr30mR, and Srmax were computed based on SI measurements (Fig 3). In the NASH group, Tmax was at 225 seconds (shortest) and 1270 seconds (longest). Although the time to Tmax tended to be longer in the NASH group, it did not differ significantly from the other groups (control = 90–180 seconds, FL = 135–270 seconds). Sr30mR in the control, FL, and NASH group was 95.43%, 97.23%, and 97.66%, respectively, and the difference was not significant. Srmax was significantly larger in NASH rabbits than the other two groups ($P < .01$).

Histological Analysis

In the control rabbits, histological study revealed no anomalies in the NAFLD activity score (Fig 4). This score ranged from 2 to 4 in FL rabbits; none of the specimens from the FL and