Paradoxical correlation between mrp2 expression and fibrosis

N. Tsuda[1], and O. Matsui[2]
[1]Medical Affairs, Bayer Yakuhin, Ltd., Osaka, Osaka, Japan, [2]Department of Radiology, Kanazawa University Graduate School of Medical Science, Kanazawa, Ishikawa, Japan

Introduction

We previously reported that the organic anion transporting polypeptide (oatp1: related to the hepatic uptake of Gd-EOB-DTPA) and multidrug-resistant protein (mrp2: related to the Gd-EOB-DTPA elimination via bile) expressions in various hepatocarcinogenesis-related hepatocellular nodules induced in rat livers were sequentially changed during the progression of hepatocellular carcinogenesis: 1) oatp1 expression was decreased in accordance with malignancy of the nodules, 2) mrp2 expression first increased in the cirrhotic liver and hyperplastic nodules, and then decreased from early HCC to moderately differentiated HCC [1]. In that study, the first step of hepatocarcinogenesis was the cirrhotic liver induced with thioacetamide; therefore, there is no information on other benign lesions (such as nonalcoholic steatohepatitis: NASH) detected in the surrounding liver parenchyma of HCC. NASH, a progressive form of fatty liver disease associated with lobular inflammation, necrosis, and perisinusoidal fibrosis developing liver cirrhosis is now considered one of the most common forms of chronic liver disease. It was reported that Gd-EOB-DTPA-enhanced MRI would be useful to assess the progress of liver fibrosis in NASH, because the Tmax (the time of maximum relative enhancement) and T1/2 (elimination half-life of relative enhancement) after Gd-EOB-DTPA injection significantly correlated with the fibrosis rate [2]. However, it remains unclear how the transporter expression changes in NASH, whether there are some differences in the transporter expression between NASH and cirrhosis, and whether the change in the transporter expression actually reflects the signal profile on the Gd-EOB-DTPA-enhanced MRI. In this study, we compared the transporter (oatp1 and mrp2) expression and signal profile on the Gd-EOB-DTPA-enhanced MRI between NASH and cirrhotic liver induced in rats, and investigated the correlation of the transporter expression and fibrosis rate in both diseases.

Methods

Thirty-six male Wistar rats aged 7 weeks were divided into three groups of twelve. The rats in the cirrhosis group were received 0.03% thioacetamide solution as drinking water for 12 weeks to induce liver cirrhosis (TAA group) and were divided into 2 subgroups as follows: Group 1 for MRI (n=7) and Group 2 for polymerase chain reaction (PCR, n=5). The rats in the NASH group were fed a choline-deficient diet for 7 or 10 weeks at 15 g/day and were divided into 2 subgroups as above. The rats in the control group were fed normal water and pellets and also divided into 2 subgroups. After MR imaging (Group 1) or liver dissections (Group 2), all livers of each group were stained with hematoxylin-eosin and Sirius red to confirm liver cirrhosis. Computer-assisted fibrosis analysis was performed using the WinROOF image processing software. **Magnetic Resonance Imaging:** Rats were anesthesthetized with 40 mg/kg intraperitoneal pentobarbital sodium and 0.5 g/kg subcutaneous urethane. Rats were imaged with 2D-FLASH (TR/TE = 101/2.9 msec, FA 90º) using a 1.0 T clinical imager. The FOV was 180 × 90 mm with a matrix of 256 × 128. Slice thickness was 5.5 mm and slice number was 12. The excitation number was 1 and the actual acquisition time was 6.4 seconds. Gd-EOB-DTPA (Primovist®: 0.025 mmol Gd/kg) was manually injected into the tail vein using a microsyringe. Sixteen image acquisitions were taken at intervals of 12sec for 3 min. Subsequently, seven MR images were acquired 5, 10, 15, 20, 30, 45 and 60 min after injection. The signal intensity (SI) of the liver was measured for each MR image, and relative enhancement (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 Gd-EOB-DTPA injection.

Laser capture microdissection (LCM), RNA extraction, and RT-PCR: Five rats of each group were used for reverse transcription polymerase chain reaction (PCR) examination. HistoGene LCM Frozen Section Staining Kit™ (Takara Bio Inc.) was used for LCM staining. The PixCellTM II LCM system™ (Molecular Devices Co.) was used to microdissect hepatocytes from unstained slides. Extracted mRNA was reverse-transcribed to generate cDNA, which was subjected to reverse transcription with a PrimeScriptTM RT regent kit™ (Takara Bio Inc.). After reverse transcription, PCR amplification was performed using Thermal Cycler Dice TP800 (Takara Bio Inc.) and a SYBR® Premix Ex TaqTM II Kit™ (Takara Bio Inc.), which is a real-time PCR-specific reagent by the intercalator method. By this technique, we compared transporter (oatp1 and mrp2) expression in the TAA, NASH and control groups.

Results

Histological findings: Liver cirrhosis was induced in the TAA group; severe fibrosis resulting in the formation of pseudolobules was induced in the whole liver in the TAA group. Perisinusoidal fibrosis in zone 3 (centrilobular area) was observed in the NASH groups and bridging fibrosis with nodular remodeling were observed in the 10 week-treated NASH group, whereas there was slight fibrosis (limited to perivenous connective tissue and Glisson's capsule) in the control group. A diffuse macrovesicular steatosis (> 66 %) was observed in the NASH groups, and a lobular inflammation, which includes polynuclear leukocytes, was also induced.

Magnetic Resonance Imaging: The time courses of relative enhancement of the liver are shown in Figure 1. Signal enhancement of the liver in the TAA group was weak in comparison with the other groups, and the relative enhancement of the TAA group was significantly lower than that of the control group (0.2 minutes: $p < 0.05$, 0.4-10 minutes: $p < 0.01$), NASH 7 week-group (0.4-60 minutes: $p < 0.01$), and NASH 10 week-group (0.4-1 minutes: $p < 0.05$, 1.2-60 minutes, $p < 0.01$).

Transporter expressions and liver fibrosis: The oatp1 expression of each group was as follows: 2.17 ± 0.71 (TAA group), 1.98 ± 0.54 (NASH 7 week-group), 2.59 ± 0.96 (NASH 10 week-group), and 2.58 ± 0.35 (control group). There was no significant difference in the oatp1 expression among each group ($p > 0.05$). The mrp2 expression of each group was as follows: 3.37 ± 1.04 (TAA group), 1.01 ± 0.21 (NASH 7 week-group), 0.74 ± 0.07 (NASH 10 week-group), and 1.00 ± 0.27 (control group). The mrp2 expression of the TAA group was significantly higher than that of the other groups ($p = 0.0011$, versus the control group; $p = 0.0011$, versus the NASH 7 week-group; $p = 0.0005$, versus the NASH 10 week-group); whereas, there was no significant difference among the control and NASH groups. Comparing the fibrosis rate and oatp1 expression, there was no significant correlation between both (NASH group: $r = 0.06$, TAA group: $r = 0.54$); whereas, a significant correlation was found between the fibrosis rate and mrp2 expression (NASH group: $r = 0.91$, $p < 0.01$; TAA group: $r = 0.85$, $p < 0.01$) (Fig 2). Paradoxical correlation between the fibrosis rate and mrp2 expression was shown in the NASH and TAA groups (i.e. negative correlation in the NASH group and positive correlation in the TAA group).

Conclusions: There was no significant difference in the oatp1 expression between the cirrhosis and NASH groups; whereas, the mrp2 expression in the TAA group increased in comparison with the NASH and control groups. In addition, there was a paradoxical correlation between the fibrosis rate and mrp2 expression. The signal enhancement on Gd-EOB-DTPA-enhanced MRI would reflect the transporter expression in NASH and cirrhosis.

Fig 1

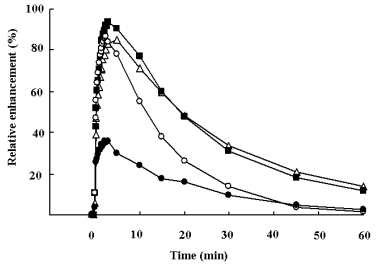
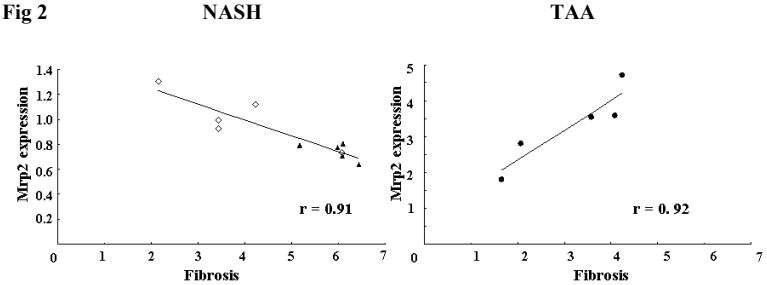


Fig 2



●: TAA, ■: NASH 10w, △: NASH 7w, ○: Control

[1]N Tsuda, et al. J Gastroenterol Hepatol. 2010; in press. [2] N Tsuda, et al. Eur J Radiol. 2010 Jan;73(1):137-42. Epub 2008 Nov 20.