Magnetic Resonance Imaging 34 (2016) 724–729

Contents lists available at ScienceDirect

Magnetic Resonance Imaging

journal homepage: www.mrijournal.com

Original contribution

Gd-EOB-DTPA-enhanced-MR imaging in the inflammation stage of nonalcoholic steatohepatitis (NASH) in mice

Tomomi Yamada [a], Atsushi Obata [a], Yuto Kashiwagi [a], Takemi Rokugawa [a], Shuuichi Matsushima [b], Tadateru Hamada [c], Hiroshi Watabe [d], Kohji Abe [a,*]

[a] Department of Drug Metabolism and Pharmacokinetics, Research Laboratory for Development, Shionogi & Co., Ltd., Osaka 561-0825, Japan
[b] Department of Drug Safety Evaluation, Research Laboratory for Development, Shionogi & Co., Ltd., Osaka 561-0825, Japan
[c] Department of Obesity and Metabolic Diseases, Discovery Research Laboratory for Innovative Frontier Medicines, Shionogi & Co., Ltd., Osaka 561-0825, Japan
[d] Division of Radiation Protection & Safety Control, Cyclotron and Radioisotope Center, Tohoku University, Sendai 980-8578, Japan

ARTICLE INFO

Article history:
Received 3 August 2015
Revised 15 January 2016
Accepted 8 March 2016

Keywords:
Dynamic contrast-enhanced-magnetic resonance imaging
Gadolinium-ethoxybenzyl-diethylenetriamine pentaacetic acid
Nonalcoholic steatohepatitis
Mouse
Methionine-choline-deficient (MCD) diet

ABSTRACT

Objective: The purpose of this study is to investigate the correlation between the liver kinetics of gadolinium-ethoxybenzyl-diethylenetriamine pentaacetic acid (Gd-EOB-DTPA) and liver histopathology in a mouse model of NASH by using dynamic contrast-enhanced MRI.
Materials and methods: Twenty male C57/BL6 mice aged 8 weeks were fed a methionine–choline-deficient (MCD) diet for 2, 4 and 6 weeks (MCD groups: MCD 2w, 4w, or 6w). Gd-EOB-DTPA-enhanced MR imaging of the liver was performed at 2, 4 and 6 weeks after the MCD feeding. The signal intensity of the liver was obtained from dynamic MR images and relative enhancement (RE), and the time to maximum RE (T_{max}) and half-life of elimination RE ($T_{1/2}$) were calculated. After MRI scan, histopathological scores of hepatic steatosis and inflammation and blood biochemistry data, such as aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels, were obtained.
Results: Plasma AST and ALT levels were significantly increased in mice fed MCD. Histopathological scores indicated that steatohepatitis progressed with the MCD feeding period from 2 to 6 weeks, but significant fibrosis was observed only in mice fed MCD for 6 weeks. Gd-EOB-DTPA-enhanced MRI showed that T_{max} was significantly prolonged in the livers of the 6-week group compared to the control group (control, 4.0 ± 0.7 min; MCD 6w, 12.1 ± 1.6 min), although there was no alteration in the 2- and 4-week groups. $T_{1/2}$ was significantly prolonged in mice fed MCD for 4 and 6 weeks compared to the control group (control, 19.9 ± 2.0 min; MCD 4w, 46.7 ± 8.7 min; MCD 6w, 65.4 ± 8.8 min). The parameters of Gd-EOB-DTPA kinetics (T_{max} and $T_{1/2}$) in the liver were positively correlated with the liver histopathological score (steatosis vs T_{max}, rho = 0.69, $P = 0.0007$; inflammation vs T_{max}, rho = 0.66, $P = 0.00155$; steatosis vs $T_{1/2}$, rho = 0.77, $P < 0.0001$; inflammation vs $T_{1/2}$, rho = 0.73, $P = 0.0003$).
Conclusions: The liver kinetics of Gd-EOB-DTPA correlated well with the inflammation score in the mouse model of NASH, suggesting the possibility of detecting the steatohepatitis stage without fibrosis by Gd-EOB-DTPA-enhanced MR imaging.

1. Introduction

Nonalcoholic fatty liver disease (NAFLD) is a major global public health problem. NAFLD is the most common hepatic disorder in Western countries and its prevalence is rising worldwide together with epidemics of obesity and type 2 diabetes mellitus [1–3]. NAFLD encompasses a range of hepatic injuries from relatively benign simple steatosis to serious nonalcoholic steatohepatitis (NASH), which can progress to liver cirrhosis and hepatocellular carcinoma [1–3]. Therefore, it is necessary to detect early stages of NASH and initiate treatment as soon as possible. The development of oxidative stress, mitochondrial dysfunction, and an increase in pro-inflammatory cytokine production are important features characterizing NASH pathology [2,4–6]. Consequently, NASH progression is mediated by an inflammatory process that causes hepatocellular damage and fibrosis. Therefore, it is important to detect liver injury induced by inflammation before the onset of fibrosis.

From the clinical viewpoint, liver biopsy is the gold standard for the diagnosis and staging of NAFLD/NASH. However, liver biopsy is an invasive procedure that is difficult to perform repeatedly. In addition, biopsy has several limitations for diagnosis due to sampling

* Corresponding author at: Department of Drug Metabolism & Pharmacokinetics, Research Laboratory for Development, Shionogi & Co., Ltd., 3-1-1 Futaba, Toyonaka, Osaka 561–0825, Japan. Tel.: +81 6 6331 7397; fax: +81 6 6332 6385.
E-mail address: kohji.abe@shionogi.co.jp (K. Abe).

http://dx.doi.org/10.1016/j.mri.2016.03.009
0730-725X/

variability and subjective semiquantitative grading [7,8]. Definitive diagnosis of early-stage NASH and repetitive assessments are required to evaluate disease progression and treatment efficacy. Therefore, there is a need for a non-invasive method for reliable diagnosis in the early stage of NASH.

Gadolinium-ethoxybenzyl-diethylentriamine pentaacetic acid (Gd-EOB-DTPA), which is a hepatobiliary contrast agent for magnetic resonance imaging (MRI), is widely used for diagnosis of liver tumors and focal lesions [9–11]. After administration, Gd-EOB-DTPA specifically accumulates in hepatocytes, which increases the signal intensity of normal liver parenchyma on T1-weighted images, and then is excreted into the bile [9–11]. Recently, Gd-EOB-DTPA-enhanced MRI has been proposed as a promising method for the characterization of liver function and staging of liver fibrosis in patients as well as NASH animal models [12–15]. However, the potential for diagnosis of early-stage NASH by Gd-EOB-DTPA-enhanced MRI has not been investigated.

The kinetics of Gd-EOB-DTPA in the liver is regulated by various hepatic transporters [16–19]. The signal enhancement after Gd-EOB-DTPA administration has been linked to transporter expression in cirrhotic livers and hepatocellular carcinomas [20,21]. Significant hepatocellular damage occurs due to oxidative stress and chronic inflammation of NASH impaired hepatic transporter function and expression [22–25]. Therefore, in the inflammatory stage of NASH before the onset of fibrosis, the uptake and excretion of Gd-EOB-DTPA by hepatocytes may change.

The purpose of this study is to investigate the correlation between the liver kinetics of Gd-EOB-DTPA and liver histopathology in a mouse model of NASH by using dynamic contrast-enhanced (DCE)-MRI.

2. Materials and methods

2.1. Animal model

All experimental protocols were approved by the Institutional Animal Care and Use Committee of Shionogi Research Laboratories. We used twenty 8-week-old specific pathogen-free male C57BL6/J mice, weighing 21 ± 1 g on average, purchased from CLEA Japan, Inc. (Shizuoka, Japan). The mice were randomly allocated into four groups (each group $n = 5$). Three groups were fed a methionine–choline-deficient (MCD) diet (Dyets, Bethlehem, PA, USA; #518,810) for 2, 4, or 6 weeks (MCD groups: MCD 2w, 4w, or 6w). MCD-fed male C57BL/6 mice have been shown to develop the histopathological features of NASH, such as steatosis, inflammation, and fibrosis [26]. As a control, one group received a standard diet (control group).

During the entire study period, the mice were maintained under standard conditions. They were allowed free access to chow and tap water and housed in a temperature-controlled room maintained on a 12-h light/dark cycle with lights on at 0800 h.

2.2. Magnetic resonance imaging (MRI)

All MRI examinations were performed with the mice under anesthesia with isoflurane/air using 1.0–2.0% through a face mask with respiratory monitoring. Core body temperature was monitored with a rectal fiber-optic probe (SA Instruments) and maintained at 37.0 ± 0.5 °C by blowing warm air into the magnet.

All MRI measurements were performed with a Varian MRI System 7 T/210 (Agilent, Palo Alto, CA, USA) controlled by Varian's VNMRJ software. A 63-mm volume coil was used for radiofrequency (RF) transmission and reception. DCE-MRI measurements were performed using T1-weighted gradient echo sequence without respiratory triggering. The parameters were as follows: repetition time/echo time, 39.06/1.42 milliseconds; flip angle, 36 degrees; field of view, 40 × 40 mm^2; matrix, 128 × 128; number of slices, 8; slice thickness, 2 mm without gap. Two minutes after the start of the scan, Gd-EOB-DTPA (0.025 mmol Gd/kg body weight) was injected into the tail vein via a 27-gauge indwelling needle connected to an extension tube and 1.0-mL syringe. In total, 135 scans including 6 pre-contrast measurements were taken at intervals of 20 s for up to 45 min.

2.3. Biochemical and histopathological analysis

After MRI study, the mice were sacrificed by exsanguination with a heparin-coated syringe under isoflurane anesthesia. Plasma was collected and assayed for the levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT). The left lateral lobes were fixed in phosphate-buffered 10% formalin, and the sections were stained with hematoxylin and eosin (HE) or Sirius-red. Steatosis, inflammation and fibrosis in the liver were comprehensively assessed by two pathologists based on severity and size of the lesion. Histopathological scores ranged from 0 to 4. (Normal, 0; Minimal, 1; Mild, 2; Moderate, 3; Marked, 4).

2.4. Data analysis

Region of interest (ROI) was set at whole liver region on one slice image because homogeneous steatosis was observed on liver tissue in this NASH mouse model. Breathing correction was not performed because the outline of the liver can be distinguished on the pre-contrast images of DCE-MRI without breathing correction (as shown in Fig. 3). SI values of the liver were measured for each MR image, and relative enhancement (RE) was calculated using the following equation:

$$RE(t) = [(SI(t) - SI(0))/(SI(0))] \times 100(\%), \quad (1)$$

where $SI(t)$ is the SI of the liver after injection of Gd-EOB-DTPA, $SI(0)$ is the average pre-contrast SI. The time course of liver RE was fitted using an empirical mathematical model (EMM) [27]:

$$RE(t) = \begin{cases} 0 & 0 \le t < t_0 \\ A \cdot \left[1 - e^{-\alpha(t-t_0)}\right]^q \cdot e^{-\beta(t-t_0)} & t_0 \le t \end{cases} \quad (2)$$

where A is the upper limit of the RE, α is the rate of contrast uptake (min^{-1}), β is the rate of contrast washout (min^{-1}), q is a parameter related to the slope of early uptake, and t_0 is the rise time point (min). The time to maximum RE (T_{max}) and half-life of elimination RE ($T_{1/2}$) were calculated from the fitting curve. In addition, T_{max} and $T_{1/2}$ maps were obtained by calculation of those parameters on a pixel-by-pixel basis.

2.5. Statistical analysis

All data are expressed as means ± SE. The differences in plasma AST and ALT levels, T_{max} and $T_{1/2}$ of the time course of RE in the liver in each group were determined by Dunnett's multiple comparison test. The differences in histopathological scores were determined by the Steel–Dwass multiple comparison test. Spearman's rank correlation test was used to evaluate the correlation between the results of DCE-MRI and liver histopathological scores. $P < 0.05$ was considered statistically significant.