MR Imaging of Hepatic Injury in the LEC Rat under a High Magnetic Field (7.05 T)

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(Received 23 July 1998/Accepted 20 November 1998)

ABSTRACT. Visualization of copper-induced hepatitis (CuH) in LEC rats was performed by using an MRI apparatus equipped with a magnet producing a high magnetic field of 7.05 T. When three groups of LEC rats (6–16 [pre-hepatitis], 15–26 [acute hepatitis] and 40–77 [chronic hepatitis] weeks old) were examined by MRI under T2-weighted imaging conditions which are suitable for the diagnosis of human hepatitis, hypointense MR images of the livers were, as a whole, obtained in all groups, suggesting that these conditions were not adequate for imaging of CuH of LEC rats. The shortening of the T1 and T2 relaxation times of livers due to an excess amount of paramagnetic ions under the high magnetic field was responsible for the lowering of MR signal intensities of the livers, especially those of 15 to 26-week old rats showing acute hepatitis. However, theoretical calculation of the MR signal intensities using the T1 and T2 relaxation times of the livers indicated that their imaging might be possible under proton density-weighted conditions even with a high magnetic field. Experimental results showed that hepatic injury was visualized as hyperintense regions in the MR image of the liver in the acute-phase rat. —Key words: hepatitis, LEC rat, MRI, T1 and T2 relaxation times.

The LEC rat is known to accumulate copper in its liver and suffers from acute hepatitis during 15–26 weeks of age [12, 16, 21]. Therefore, this rat is considered a good animal model for studying copper-induced acute and chronic hepatitis as well as hepatocellular carcinoma (HCC). In a previous study, we reported that magnetic resonance image (MRI) with a magnet producing a high magnetic field of 7.05 T (higher than that of usual human MRI, 0.2–2 T) was applicable for the diagnosis of copper-induced HCC in LEC rats [1]. In T1-weighted MR imaging of the 12-month-old LEC rat liver, hyperintense regions at the circumference of hepatic veins were diagnosed as HCC.

The next step is to apply this imaging technique to diagnose copper-induced hepatitis in LEC rats. In the human liver, hepatitis is detected as hyperintense regions in T2-weighted MR imaging [10, 13]. However, if MR imaging of the liver containing high amounts of paramagnetic ions (40–50 times higher than those of normal rats) is carried out with a high magnetic field, the T2-weighted MR signal intensities may decrease to give hypointense MR images because of the shortening of the T1 and T2 relaxation times [1, 15, 22], though the theory of magnetic resonance suggests that MR imaging under a high magnetic field is favored over imaging under a low magnetic field for small animals such as mice and rats [1, 8, 11, 20]. Actually, hypointense MR images of the livers were observed under the T2-weighted conditions in all groups. Theoretical calculation of the MR signal intensities under three sets of conditions (T1-, T2- and proton density-weighted conditions) using the T1 and T2 relaxation times obtained experimentally from the livers of three groups at 7.05 T proved that MR imaging of the livers was impossible in all groups under the T2-weighted conditions. However, this result also suggested that MR imaging of the livers might be possible under proton density-weighted conditions. Therefore, in the present study we examined whether MR imaging at 7.05 T under proton density-weighted conditions was adequate to image copper-induced hepatitis in LEC rats.

MATERIALS AND METHODS

Animals: Male LEC and male Wistar rats were maintained under conventional conditions at the Experimental Animal Facility of the Graduate School of Veterinary Medicine of Hokkaido University. They were fed a regular diet and water ad libitum in an air-conditioned animal room at 22 ± 3°C with a relative humidity of 55 ± 5% [1, 8, 11, 21]. LEC rats were divided into three groups: pre-hepatitis (6 to 16 weeks old, n=6), acute hepatitis (15 to 26 weeks old, n=6) and chronic hepatitis (40 to 77 weeks old, n=6). Severe jaundice, as a symptom of fulminant hepatitis, was judged by ocular inspection [21]. Wistar rats (16 to 40 weeks old, n=6) were chosen as a reference standard.

MRI: Proton MR images at a 7.05 T were obtained as previously described [1, 8, 11]. After anesthetization, each rat was positioned supine in the probe running parallel to z-axis of the magnet, and fixed to a cradle using adhesive tape to minimize artifacts due to respiratory motions. After adjusting the coils, MR images of the transversal sections of the hepatic region were taken using a spin echo (SE) sequence with four acquisitions and pulse width of 4 kHz. The pulse sequence conditions for T1-, T2- and proton density-weighted imagings were repetition time (TR)/echo time (TE) = 500/20, 2,000/80 and 2,000/20 ms, respectively
Measurement of $T_1$ and $T_2$ relaxation times of the liver: Some rats were killed with an overdose of pentobarbital sodium solution (200 mg/kg body weight, i.p.) after imaging. Livers were removed and blood washed out with physiological saline. Liver sections were cut off to a volume of $2 \times 3 \times 15$ mm$^3$ from the left lobe. The sections were put into Pyrex tubes (5 mm in diameter) for nuclear magnetic resonance (NMR) spectroscopy. The $T_1$ and $T_2$ relaxation times were measured on a Bruker 7.05 T superconducting magnet (10 cm vertical-bore) equipped with an MSL-300 system. Using localized volume selective spectroscopy (VOSY) [3, 17], the MR signal intensity was judged to arise mainly from water protons.

$T_1$ and $T_2$ values: On the basis of the $T_1$ and $T_2$ values measured above, the MR signal intensities in three pulse sequences ($T_1$, $T_2$- and proton density-weighted), $I$, were calculated with the Bloch equation [5],

$$ I = N \left[1 - \exp\left(-\frac{TR}{T_1}\right)\right] \exp\left(-\frac{TE}{T_2}\right) $$

where $N$ = proton density, $TR$ = repetition time, $TE$ = echo time, $T_1$ = $T_1$ relaxation time, $T_2$ = $T_2$ relaxation time. The flow term in equation (1) was neglected under the conditions used in the present study. To evaluate the relative contributions of $T_1$, $T_2$- and proton density-weightings to MR images, the parameter weighting indexes were also calculated according to the method of Elsner [5].

$$ T1WI (\%) = \frac{S_{T1}}{S_{T1} + S_{T2} + S_N} \times 100 $$

$$ T2WI (\%) = \frac{S_{T2}}{S_{T1} + S_{T2} + S_N} \times 100 $$

where $S_{T1}$ = $(TR/T_1)\left[1 - \exp\left(-TR/T_1\right)\right]$, $S_{T2}$ = $TE/T_2$, and $S_N$ = 1, and are called 'fractional sensitivities' of $I$ with respect to $T_1$, $T_2$, and $N$.

Measurement of metal concentrations in the liver: The concentrations of copper and iron in the liver were measured by flameless atomic absorption (FAA) spectrometry using a SHIMADZU AA 630–12 spectrometer (Kyoto, Japan) [18].

Histological examination: Liver sections were prepared for histological examination from the right lobe. Tissue samples were fixed with 4% paraformaldehyde solution in 0.1 M phosphate buffer (pH 7.3) overnight at 4°C. Paraffin sections (3 μm) prepared by routine procedure were stained with hematoxylin-eosin [21].

RESULTS

$T_1$ and $T_2$ relaxation times: Table 1 shows the $T_1$ and $T_2$ relaxation times of the livers in the pre-, acute and chronic hepatic phases of LEC rats and the liver of the Wistar rat at 7.05 T. No significant difference in $T_1$s between the livers of pre- and chronic hepatic phases of LEC rats and the liver of the Wistar rat was observed. However, $T_1$ and $T_2$ in the acute phase were significantly shorter than those of others. Engelhardt et al. reported that the $T_1$ and $T_2$ of the Wistar rat at 1.5 T were 700 and 40 ms, respectively [6]. The present study of the Wistar rat showed that $T_1$ at 7.05 T was longer than that at 1.5 T, whereas $T_2$ at 7.05 T was quite similar to that at 1.5 T. These observations were in accord with the general conclusion that $T_1$ increases depending on the magnetic field strength and $T_2$ changes little with respect to it [2].

To explain the shortening of $T_1$ and $T_2$ observed in the acute phase, we measured the concentrations of paramagnetic copper and iron ions in the three phases as well as in the liver of the Wistar rat (Fig. 1). The rapid increase in the Fe concentration that was observed in the acute phase seemed responsible for the shortening of $T_1$ and $T_2$ values.

Calculated MR signal intensities and parameter weighting indexes: Using the $T_1$ and $T_2$ values shown in Table 1, the MR signal intensities as well as parameter weighting indexes under the three SE conditions were calculated (Table 2). In general, the signal intensity ($I$) under the proton density-weighted conditions ($TR/TE = 2,000/20$ ms) gives us information about the number of protons per unit volume (N) in equation (1), which is related to the pathological tissue alterations, since the MR image may be mostly affected by NWI in this sequence. Contrary to the expectation, the effect of NWI on the MR signal intensity in this case was only about 50% (see Table 2), suggesting that the information of liver proton density at each phase could essentially not be obtained from MR imaging under these conditions. The signal intensity in Table 2 was,
therefore, expressed as I/N. Nevertheless, parameter weighting indexes gave interesting results in that the relative contribution of T1WI to the MR intensity was only 28–36% and smaller than that of NWI (41–44%) under the T1-weighted imaging conditions, whereas that of T2WI to the MR intensity was largest (60–70%) among the three indexes under the T2-weighted imaging conditions as expected.

From Table 2, it becomes clear that the proton density-weighted imaging (TR/TE = 2,000/20 ms) gave the highest MR intensities among the three sets of imaging conditions. The T1-weighted imaging (TR/TE = 500/20 ms) had intermediate intensity, and the T2-weighted imaging (TR/TE = 2,000/80 ms) gave the lowest intensity, especially in the acute hepatic phase, indicating that T2-weighted imaging at 7.05 T is not suitable for MR imaging of the livers of all groups, whereas MR imaging of the livers under the proton density weighting seems possible even at 7.05 T.

**MR imaging of LEC rat livers as well as Wistar rat liver under three SE conditions**: In accord with the theoretical results in Table 2, hypointense MR images were, as a whole, obtained from all livers including the liver of the Wistar rat by T2-weighted imaging (data not shown). MR images obtained by proton density-weighted imaging are shown in Fig. 2. Hypointense MR images were still obtained from the livers of rats in the pre- and acute phases (Fig. 2b and 2c), whereas MR images with relatively clear contrast were obtained from the liver of rat in the chronic phase as well as that of the Wistar rat (Fig. 2a and 2d). These observations agreed with the theoretical calculation in Table 2 in which MR intensities of rats in the pre- and acute phases were lower than those of rats in the chronic phase and Wistar rats.

Though the rat liver in the acute phase gave a hypointense MR image, scattered spotty hyperintense regions were observed throughout the liver tissue (Fig. 2c). We diagnosed the hyperintense regions as hepatitis-related tissue alterations. The degree of T2WI in the proton density-weighted image became largest (35%). This may be the reason why the hyperintense regions appeared in the hypointense MR image.

**Histological examination**: By macroscopic observation, there were some white nodules at the surface of liver (Fig. 3a, arrows). Microscopic changes were observed in LEC rat in which the liver showed massive and multifocal necrosis with filtration of a few inflammatory cells and giant hepatocytes. Proliferation of pseudobile ducts and increased numbers of kupper cells phagocyting were observed (Fig. 3b and 3c). These results indicated 15 to 26 week-old rats were diagnosed as fulminant hepatitis like a previous our report [21].

**DISCUSSION**

MR imaging is now widely employed as a useful technique for clinical diagnosis in veterinary medicine [1, 8, 11, 17, 20]. To obtain strong NMR signals with a high
S/N ratio from small laboratory animals, a high magnetic field is generally required \[1, 8, 11, 20\]. Several investigators have reported MR images of LEC rat livers \[1, 15, 22\]. However, they found that the MR signal intensities decreased in the hepatic phase. This phenomenon was inferred by the shortening of the T_1 and T_2 relaxation times under the high magnetic field due to the presence of excessive amounts of metal ions in the LEC rat liver. However, no T_1 and T_2 relaxation times of the LEC rat liver under a high magnetic field have been reported and, therefore, no quantification about the degree of T_1-, T_2- or proton density-weighting which a given SE pulse sequence actually possesses under the conditions of short T_1 and T_2 relaxation times and a high magnetic field has been done. Furthermore, since equation (1) means that the MR intensities depend on not only the T_1 and T_2 relaxation times but also the TR and TE times, it is necessary to study the effects of a high magnetic field and shortening of the T_1 and T_2 relaxation times on the MR intensities under the three SE conditions.

In the present study, we first measured the T_1 and T_2 relaxation times of the LEC rat livers in the pre-, acute and chronic phases and calculated their MR signal intensities. In addition, we also calculated three parameter weighting indexes (T1WI, T2WI and NWI) to quantitate the degree of T_1-, T_2- or proton density-weighting in the MR images. Experimental results demonstrated that the T_1 and T_2 relaxation times were reduced under the high magnetic field of 7.05 T (Table 1). When the Cu and Fe concentrations in pre-, acute and chronic hepatitis were measured, only the change in the Fe concentration paralleled that in the relaxation times. Therefore, we concluded that the high concentration of Fe in the acute phase was responsible for their shortening. Human acute hepatitis was visualized as hyperintense regions in the T_2-weighted images because of the changes in the T_1 and T_2 relaxation times due to the histological damage \[13\]. In acute hepatitis of LEC rats, excessive damage due to the collapse of the liver was...
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observed [16]. Calculation of the MR signal intensities using these T1 and T2 relaxation times under the three SE conditions (500/20, 2,000/80 and 2,000/20) indicated that T2-weighted MR imaging of the livers was not suitable to diagnose copper-induced hepatitis. However, the calculation also indicated that MR imaging of the livers might be possible under the proton density-weighting. In fact, scattered spotty hyperintense regions distinguishable from the others, which could be diagnosed as the hepatitis-related tissue alteration, were observed throughout the liver tissue in the acute phase, though the liver in this phase presented, as a whole, a hypointense MR image (Fig. 2c).

In our previous paper, we diagnosed the hyperintense regions around hepatic veins as HCC in T1-weighted MR images of the 12-month-old LEC rat liver [1]. The massive tissue damage due to HCC would enable T1-weighted MR imaging to visualize the HCC-related tissue alterations. The hyperintense regions are generally identified as HCC in T1-weighted MR images of the liver in the human [4, 9, 14]. We tried to image hepatic regions under the T1-weighted conditions, but those regions were not clearly distinguished from the other regions (data not shown). This was probably because alterations of the liver tissue in hepatitis are not as drastic as those in HCC.

Table 2 presents parameter weighting indexes. It shows that (1) an SE 500/20 image of the liver which is usually employed as a T1-weighted image, when performed at 7.05 T, is more proton density-weighted (41–44%) than T1-weighted (28–36%), (2) an SE 2,000/80 image which is usually employed as a T2-weighted image actually possesses much T2-weighting (60–70%), and (3) an SE 2,000/20 image, which is usually employed as a proton density-weighted image does not possess so much proton density-weighting (51–54%). Thus, when MR studies are performed on tissues containing high amounts of paramagnetic ions such as LEC rat livers at different field strengths, using different pulse sequences and timing intervals, it is necessary to determine whether the MR pulse sequence is sensitive to small changes in tissue N, T1, or T2.

ACKNOWLEDGMENTS. This research was supported in part by a Grant-in-Aid for Basic Scientific Research from the Ministry of Education, Science, Sports and Culture of Japan (No. 09556064 to M. K.). In conducting this work, the investigators adhered to The Guide for the Care and Use of Laboratory Animals, Graduate School of Veterinary Medicine, Hokkaido University.

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Fig. 3. Morphological changes of LEC rat (24 weeks old): (a) Some white nodules were macroscopically observed at the surface of liver (arrows), (b) and (c) Microscopical examination (b; × 33, c; × 132).