

Application of Diffusion Weighted Proton MRI for Evaluating Molecular Diffusion of Water and Tissue Perfusion in Diethylnitrosamine Induced Rat Liver Fibrosis

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Introduction

Chronic liver disease is a major public health problem worldwide (1). Early and accurate diagnosis of liver cirrhosis is crucial for prognostic assessment of the course of the disease. Liver biopsy, the standard for diagnosing liver fibrosis has many limitations, including invasiveness, sampling error, observer variability, and the use of categorical scoring system. Measurement of water apparent diffusion coefficient (ADC) by diffusion weighted (DW) ¹H MRI may be useful in diagnosis of cirrhosis, because increased deposition of extracellular matrix components in fibrosis may lead to decreased water ADC (2,3). DW ¹H MRI has potential to provide information about both random molecular diffusion and tissue perfusion by carefully selecting a range of low (<100 mm²/s) and high (> 100 mm²/s) *b*-factors (4). The main goal of this research was to determine the effects of hepatic fibrosis in an animal model of cirrhosis produced by diethyl nitrosamine (DEN) on molecular diffusion of water and tissue perfusion, using DW ¹H MRI.

Methods

Liver fibrosis was induced in male Wistar rats by continuous administration of 0.01% DEN via drinking water for 6 weeks (DEN group, *n* = 10) (5). The control (CON) animals received normal water over the same period (CON group, *n* = 6). MR experiments were conducted on a Varian 9.4 T, 31 cm horizontal bore system using a 6.3 cm diameter quadrature birdcage coil after 6 weeks of treatment. A 10 mm diameter tube filled with 54 mM NaCl was placed inside the coil and used as a ¹H signal intensity (SI) reference. Total, water and fat ¹H MRI were obtained with a spin-echo sequence (TR/TE = 1100/12 ms) without and with fat or water suppression using chemical shift selective (CHESS) technique (3). DW ¹H MRI was collected using a modified spin-echo sequence (TR/TE = 1100/25 ms) and 10 *b*-factors (*b* = 0, 10, 20, 30, 100, 220, 350, 600, 1000, 1600 s/mm²). Twelve transaxial slices with 1.5 mm slice thickness and 0.5 mm slice gap and 128 × 128 data points over 64 × 64 mm FOV were collected for all MRI. Respiratory gating was used to minimize the effects of motion on water ADC measurements. Mortal ischemia was produced by increasing the isoflurane to 4.5 % in medical air at 4 L/min. Respiration was monitored until it stopped, then again DWI ¹H MRI were collected for the 10 *b*-factors. After the MRI experiments, the liver was fixed for histological analysis. DW MRI SI versus *b*-factor data were fit to the following biexponential equation: $SI = SI_0 [A_f \times e^{-b \times ADC_{fast}} + (1 - A_f) \times e^{-b \times ADC_{slow}}]$, where SI is signal intensity for a given *b*-factor, ADC_{fast} and ADC_{slow} are the fast and slow ADC component which are related to tissue perfusion and random molecular diffusion of water, respectively, and A_f is the relative contribution of ADC_{fast} which is related to the relative vascular volume.

Results

Fig. 1 shows representative transaxial sections of total, water, and fat images from a DEN and CON treated rat. As shown in Table 1, water SI relative to the reference was significantly higher in DEN group (0.52 ± 0.05) compared to CON group (0.38 ± 0.07). However, fat SI relative to the reference was not significantly different in the two groups. The increase in water MRI SI with DEN treatment was consistent with a lower dry-to-wet weight ratio in DEN group compared to CON group. DW ¹H MRI data for rats in both CON and DEN groups showed biexponential plots for live animals and monoexponential plots for dead animals. As expected, ADC_{fast} was not detectable in dead animals in either group. In live animals, ADC_{fast} was lower in DEN group compared to CON group (*p* < 0.05), but A_f was higher in DEN group. ADC_{slow} was same in DEN and CON groups for live animals. Upon death ADC_{slow} did not change for DEN group but it decreased for CON group (*p* < 0.05). This resulted in a higher ADC_{slow} for DEN group compared to the CON group after death. Representative histology slides from CON and DEN treated animals are shown in Fig. 2. DEN caused focal periportal fibrosis, bridging fibrosis, multifocal bile duct proliferation, focal cholangiohepatitis, and regenerative nodules. A scatter plot of ADC_{fast} versus fibrosis score is shown in Fig. 3. ADC_{fast} was lower in the presence of liver fibrosis but a correlation between ADC_{fast} and fibrosis score was not detectable.

Discussion

It is generally believed that ADC of water decreases with liver fibrosis because of increased deposition of extracellular matrix components (3). This idea is supported by data from many previous DW ¹H MRI studies that used low *b* factors (< 500 mm²/s) and analyzed the data using a monoexponential model (3,4). In the present study, contributions of random restricted diffusion of water and tissue perfusion to ADC were separated by carefully selecting ten *b*-factors and fitting the data to a biexponential model. In contrast to previous studies, the data presented here show that liver fibrosis produced by DEN does not alter the random molecular diffusion of water because ADC_{slow} were identical for live animals in DEN and CON groups. In fact, water diffusion may be increased with fibrosis because ADC_{slow} for dead animals was higher for DEN group compared to CON group. The higher liver water content measured by both ¹H MRI and dry-to-wet weight ratio may be related to the observed increased ADC_{slow} in DEN group. Hepatic fibrosis by DEN also caused a decrease in tissue perfusion because ADC_{fast} was lower in DEN group compared to CON group. The decrease in perfusion appears to be accompanied by an increase in vascular volume because A_f was higher in DEN group compared to CON group. Similar results have recently been reported in a human study by Luciani et al. (5).

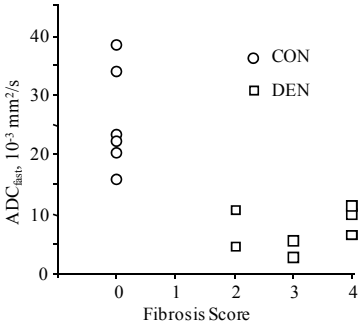


Fig. 3. Hepatic ADC_{fast} as a function of fibrosis score for CON and DEN treated rats.

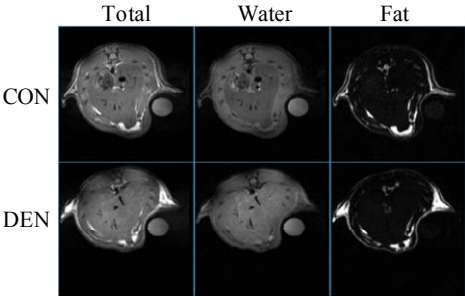


Fig. 1. Total, water and fat ¹H MRI of a CON and DEN rats.

Table 1. Effects of DEN treatment on dry-to-wet weight ratio, and parameters measured by fat and water suppressed ¹H MRI and DW ¹H MRI. Water and fat MRI SI are reported relative to the reference without fat or water suppression. ADC values are in 10⁻³ mm²/s. * *p* < 0.05

Parameter	CON	DEN
Dry to wet wt ratio	0.30 ± 0.03	0.22 ± 0.03*
Water SI	0.38 ± 0.07	0.52 ± 0.05*
Fat SI	0.051 ± 0.008	0.056 ± 0.003
A_{fast} (live)	0.35 ± 0.03	0.42 ± 0.03*
ADC_{fast} (live)	26 ± 4	10 ± 3*
ADC_{slow} (live)	0.52 ± 0.11	0.47 ± 0.05
ADC_{slow} (dead)	0.31 ± 0.04	0.46 ± 0.02*

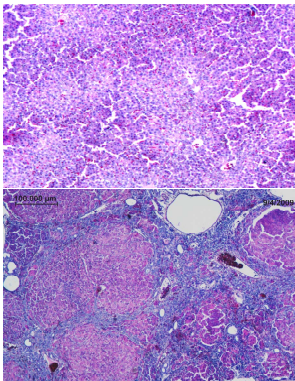


Fig. 2. H&E stained histological sections of a CON (top) and DEN (bottom) rat liver with grade 4 fibrosis showing bridging fibrosis, focal cholangiohepatitis, diffuse bile duct proliferation, large dilated cyst, inflammation and regenerative nodules.

Conclusion

DW ¹H MRI can be useful in evaluating both restricted molecular diffusion of water and tissue perfusion. The previously reported decrease in ADC with liver fibrosis (3,4) was mainly caused by a decrease in perfusion and not because of a decrease in water diffusion. Thus measurement of perfusion by DW ¹H MRI may be a useful marker for diagnosis of fibrosis. A larger study is needed to evaluate the use of DW ¹H MRI for staging of liver fibrosis.

References

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