Diffusion Tensor Imaging (DTI) in Liver Fibrosis with Minimal Confounding Effect of Hepatic Steatosis

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TARGET AUDIENCE

The target audience for this work is clinicians who are interested in the efficacy of diffusion MRI in the assessment of liver diseases.

INTRODUCTION

The apparent diffusion coefficient (ADC) and fractional anisotropy (FA) in diffusion MRI have been suggested to be potential MR parameters in the diagnosis of liver fibrosis [1]. However, recent studies demonstrated that ADC can be altered not only by fibrosis but by steatosis as well [2]. In addition to these studies that focused on the relationship between ADC and fat content in the liver, it should also be important to clarify the relationship between ADC and the severity of liver fibrosis after accounting for the potential confounding effect of steatosis. However, it is challenging to address such an issue as fatty liver is common in liver patients. The severe image artifact resulting from the use of an echo planar imaging (EPI) in diffusion MRI for rapid abdominal scans is another hindrance.

PURPOSE

To this end, the purpose of the study was to investigate the efficacy of diffusion tensor imaging (DTI) in the assessment of liver fibrosis in vivo in an experimental setting where the potential confounding effect of fat on ADC and FA can be negligible. For this purpose a suitable animal model was employed, in which a broad range of the severity of liver fibrosis was obtained and yet with minimal fat content throughout the course of the disease progression. In addition, diffusion MR images were collected in combination with a conventional spin-echo (SE) sequence instead of EPI in order to minimize image artifacts.

METHODS

The animal research protocol was approved by the IACUC. Male C57BL/6 mice were used in this study (n=36). Liver fibrosis was induced in 20 mice by an i.p. injection of ccl4 mixed with vegetable oil for 2-10 weeks [3]. There were 16 control mice. Following the MR scans, liver samples were collected for histopathologic examinations. Livers were scored for necrosis, inflammation and fibrosis (severity scores ranging 0-4).

MR data were collected on a 9.4T animal MR scanner with a transmit-only volume coil and a phased-array 4 channel surface coil (Bruker). Axial DTI images were acquired using a respiratory-gated, fat-saturated, spin-echo DTI sequence with 6 gradient directions at b=500 s/mm^2. Images at b=0 s/mm^2 were also acquired (FOV=28x22 mm^2, matrix size=256x128, TR/TE=4000/20 ms, 8 slices (1 mm-thick), 1 signal average, and bandwidth=100 kHz). For hepatic fat quantification proton MRS was performed with a STEAM sequence (TR/TE/TM=5000/2.2/3 ms, spectral width=5000 Hz, number of data points=2048, 32 signal averages, 3 voxels (1.3x1.3x1.3 mm^3)) and the resulting hepatic fat fraction (HFF = fat/(water+fat)x100) was used as a measure of hepatic fat content.

All data analyses were performed using Matlab (Mathworks Inc.). The ADC and FA were calculated according to ref. [4]. For each animal, regions of interest (ROIs) were defined on the image obtained at b=0 s/mm^2 for each slice and then the mean ADC and FA across the slices were used in the data analysis. MRS data were processed by using MRUI. HFF was calculated for each voxel and the mean HFF over the three voxels was used in the data analysis.

For pair-wise group comparisons the Mann-Whitney U test was used. For multiple pair-wise group comparisons Bonferroni correction was performed. The Spearman Rho test was used to examine correlations between the histopathologic and MRI parameters ($p < 0.05$ considered to indicate statistical significance).

RESULTS

In Fig.1, the high SNR, lack of artifacts and high resolution of the images resulting from the use of SE instead of EPI are demonstrated. Despite the presence of moderate amount of fibrosis (Fig.1(l)), fatty change (steatosis) was absent (Fig.1(k)) in the treated mouse. There were 4, 11, and 5 mice in the animal groups with fibrosis scores of 1, 2, and 3 (F1-F3), respectively. All ccl4-treated mice were grouped together as a treated group (n=20).

The mean HFFs of the two groups (Fig.2a) were both below 5.5%, and did not differ from each other (5.3±1.5 vs. 4.6±1.1 %; p=0.115). The necrosis, inflammation and fibrosis scores were all significantly higher in the treated group with respect to control (Figs.2b-2d). Nonetheless, there was no difference in ADC between the two animal groups (0.711±0.068 x10^{-3} vs. 0.718±0.095 x10^{-3} mm^2/s; p=0.911) (Fig.2e). On the other hand, FA of the treated group was significantly lower than that of control (0.552±0.050 vs. 0.586±0.013; p=0.023) (Fig.2f).

ADC was not correlated with any of HFF, necrosis, inflammation and fibrosis. FA was not correlated with necrosis and inflammation, but was positively correlated with HFF (r=0.418, p=0.011) and negatively correlated with fibrosis (r=-0.411, p=0.012).

DISCUSSION

While there was no difference in HFF, the necrosis, inflammation and fibrosis scores were all significantly elevated in the treated group. Nonetheless, ADC did not differ between the two animal groups. Together with the lack of correlations between ADC and the histopathologic parameters, therefore, ADC may not sensitively detect mild-to-moderate fibrosis. Such a poor sensitivity of ADC to the severity of liver fibrosis has also been reported previously [5]. Instead, FA was significantly lowered in the treated group. Therefore, given the comparable HFF between the two animal groups, the elevation of the histopathologic parameters, and its negative correlation with fibrosis altogether, FA may more sensitively detect the severity of liver fibrosis than ADC. In addition to the negative correlation with fibrosis, FA was positively correlated with HFF. This means that, unlike in our current animal model, such potential sensitivity of FA to both fibrosis and HFF may not be depicted, for instance, in human liver fibrosis where severe steatosis can also develop thereby compensating for the effect of fibrosis. In line with our finding, a higher FA in fatty liver patients with respect to normal patients has been reported previously [6]. The lack of correlation between ADC and hepatic fat content in our study may likely be due to the limited dynamic range of the HFF values (2.8-8.9%) and the relatively low mean HFF value (4.92±1.32 %) of our animals. This may, in turn, indicate that FA is more sensitive to fatty change than ADC as it is to fibrosis. In this case, our study also suggests that altered FA in the liver may also need to be carefully interpreted in the presence of fatty liver. Given the intermediate b-value of 500 s/mm^2 in this study, our results may have been confounded by blood perfusion [7]. However, the use of SE instead of EPI in our study also allowed relatively higher image resolution than that which is typically obtained in a single-shot EPI readout in the liver. Consequently, the effect of blood perfusion against pure water diffusion should be reduced [7].

CONCLUSION

At b=0 and 500 s/mm^2, FA may be more sensitive to mild-to-moderate liver fibrosis than ADC. In addition to ADC as demonstrated elsewhere, FA may also be sensitive to hepatic fat content, and therefore need to be carefully interpreted in the presence of fatty liver.

REFERENCES

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ACKNOWLEDGEMENTS

This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2009-0077642, 2010-0002896, 2013R1A1A2013516).

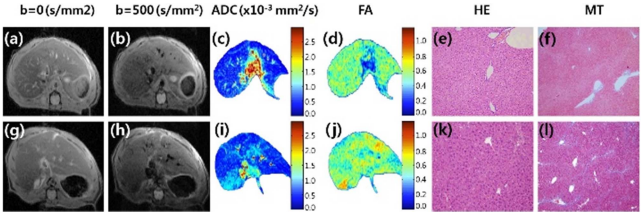

Figure 1. *Representative liver images at b=0 and 500 s/mm^2, and ADC- and FA-maps along with the HE- and MT-stained liver sections for a control (a-f) and a treated (g-l) mice.*

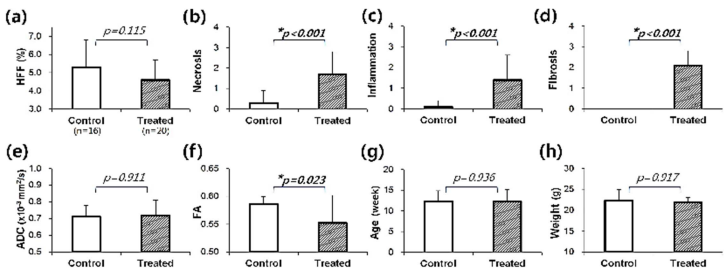

Figure 2. *Comparison between the control and the treated animal groups*