INTRAVOXEL INCOHERENT MOTION (IVIM) ANALYSIS OF LIVER FIBROSIS IN AN EXPERIMENTAL MOUSE MODEL

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NTRODUCTION

Liver fibrosis is a common response to chronic liver injury with high morbidity and mortality[1]. Early diagnosis of liver fibrosis could facilitate early interventions and treatments, preventing its progression to cirrhosis[2]. Recently, diffusing imaging has been shown to be promising in characterization of liver fibrosis[3,4]. Given the relatively high blood volume fraction in liver[5], perfusion can contribute significantly to the diffusion measurements significantly because of the incoherent motion of blood in pseudorandom capillary network at macroscopic level. Intravoxel incoherent motion (IVIM) analysis was developed previously to quantify the diffusion and perfusion effects separately[6]. Blood perfusion in chronic liver disease has also been recognized as an important marker of liver fibrosis[7]; therefore, IVIM analysis may be more advantageous and sensitive than conventional diffusion imaging in characterizing liver fibrosis. In this study, we aim to characterize changes in molecular water diffusion, blood microcirculation, and their contributions to the apparent diffusion changes using IVIM analysis in an experimental mouse model of liver fibrosis.

METHODS

Animal Preparation: Male adult C57BL/6N mice (22-25g; *N*=12) were prepared. Liver fibrosis was induced by subcutaneous injection of 1:3 mixture of carbon tetrachloride (CCl_4) in olive oil at a dose of 4μL/g of body weight twice a week for 4 weeks[8]. The twice-weekly dosing can induce early stages of liver fibrosis and established fibrosis after 2 and 4 weeks of CCl_4 administration, respectively, in rodents[8]. Diffusion MRI was performed in animals 1 day before, 2 and 4 weeks after CCl_4 administration. ***MRI:*** All MRI experiments were performed on a 7T Bruker MRI scanner using a 38-mm quadrature RF coil. Under inhaled isoflurane anaesthesia, the animal was kept warm under circulating water at 37°C. DTI was performed in one axial slice covering the liver. The respiratory-gated DW images were acquired using single-shot SE-EPI with 8 b-values (0,50,100,200,500,1000,1500,2000s/mm^2) and single direction, TR≈2000ms, TE=40ms, δ/Δ=3.1/27ms, acquisition matrix=64×64, spatial resolution=0.78×0.78×2mm^3, NEX=10. DTI protocol was also employed using TE=32ms, δ/Δ=2.6/20ms, b-values=0,1000s/mm^2, 6 diffusion gradient directions, and all other parameters were the same as the DWI sequence above. The DTI acquisition was repeated twice. ***Data Analysis:*** DW images were first co-registered using AIR5.2.5. To examine the individual contributions of molecular water diffusion and blood microcirculation to the apparent diffusion changes, true diffusion coefficient (D), blood pseudodiffusion coefficient (D*), and perfusion fraction (f) were estimated using a least-square nonlinear fitting in Matlab by fitting the DW signal decay to the IVIM bi-compartmental model on a pixel-by-pixel basis as follows: $SI/SI_0=(1-f)\times\exp(-bD)+f\times\exp(-bD^*)$. Apparent diffusion coefficient (ADC) and fractional anisotropy (FA) maps were generated from DTI data using DTIStudio[9]. A ROI was defined to encompass a large homogeneous liver region for IVIM analysis and DTI measurements. One-way ANOVA with Tukey's multiple comparison test was employed to compare the measurements between different time points of liver fibrosis, with $p<0.05$ considered as statistically significant. ***Histology:*** Animals were sacrificed immediately after MR examinations. Liver specimens were fixed in formalin, embedded in paraffin, sectioned and examined by light microscopy after standard H&E and Masson's trichrome staining.

RESULTS

Fig. 1 shows the D, D* and f values at different time points after CCl_4 insult based on the IVIM analysis of DW images with multiple b-values. Fig. 2 shows the typical ADC and FA maps (computed from DTI data) of liver 1 day before, 2 and 4 weeks after CCl_4 insult. ADC and FA values at different time points are shown in Fig. 3, which were in agreement with our previous DTI study of an experimental rat fibrosis model[10]. Fig. 4 shows the typical H&E and Masson's trichrome staining of normal liver, and livers at 2 and 4 weeks after CCl_4 insult. Compared with normal liver, collagen deposition and intracellular fat vacuoles were consistently observed in livers with CCl_4 insult. Cell necrosis/apoptosis was evident in liver with 2-week CCl_4 insult, while collagen deposition was more pronounced in liver with 4-week CCl_4 insult.

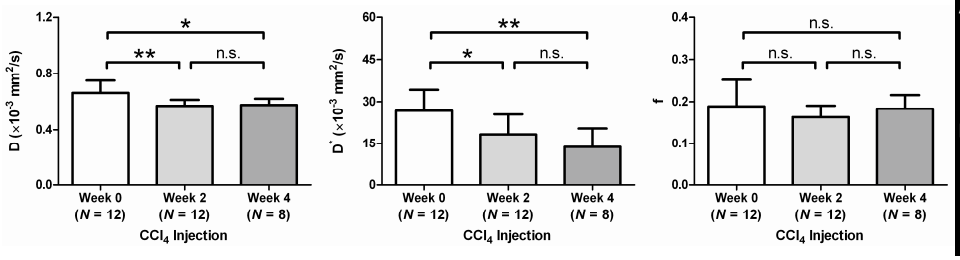

FIG. 1. D, D* and f values of animals at 0, 2 and 4 weeks after CCl_4 insult as computed by IVIM analysis of DW images. One-way ANOVA was performed with * for $p<0.05$, ** for $p<0.01$ and n.s. for insignificance.

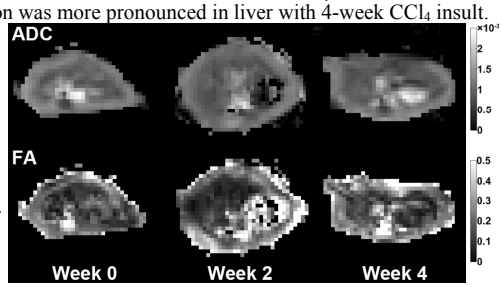

FIG. 2. ADC and FA maps from one animal at 0, 2 and 4 weeks after CCl_4 insult as computed from DTI data.

DISCUSSIONS AND CONCLUSIONS

True diffusion coefficient D and ADC were observed to be decreasing gradually after CCl_4 insult, likely due to the increased extracellular collagen deposition and increased intracellular fat droplets during the progression of liver fibrosis (Figs. 4b,c). Change in ADC could also be associated with the decreased blood perfusion, which has also been suggested in several DWI studies[11,12]. Reduced blood pseudodiffusion coefficient D* likely resulted from the inability of increased arterial flow triggered by intrahepatic portal hypertension to compensate for the reduced portal flow[13]. Perfusion fraction f was observed to be unchanged after CCl_4 insult, which was in agreement with previous IVIM studies of liver cirrhosis[14,15]. This could be explained by the hepatic arterial vasodilatation in response to reduced portal flow[16]. It is worth noting that the percentage change in D* at 2 weeks after CCl_4 insult (27%) was higher than that in FA (25%), ADC (17%) and D (12%) at 2 weeks after insult, indicating that D* could provide higher sensitivity in detecting early liver fibrosis. The experimental results from this study showed that both molecular water diffusion and blood microcirculation contribute to the alteration in apparent diffusion changes observed in liver fibrosis. IVIM analysis may be valuable for characterizing liver fibrosis at early phase and monitoring its progression.

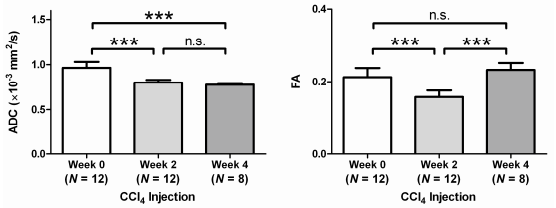

FIG. 3. DTI-derived ADC and FA values of animals at 0, 2 and 4 weeks after CCl_4 insult. One-way ANOVA was performed with *** for $p<0.001$ and n.s. for insignificance.

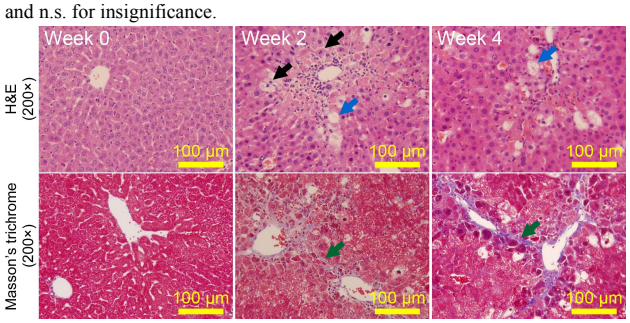

FIG. 4. Typical H&E staining (×400) of (**a**) normal liver, and livers subjected to (**b**) 2-week and (**c**) 4-week CCl_4 twice-weekly administration. Collagen deposition (green arrows), fat vacuoles (blue arrows), and cell necrosis/apoptosis (black arrows) were observed in the insulted livers.

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