Increasing diffusion time improves in vivo DWI sensitivity to liver fibrosis

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INTRODUCTION: Hepatic fibrosis, which occurs in response to chronic liver injury from many causes such as hepatitis and alcohol intoxication [1], was historically regarded as irreversible process. In recent years, it is considered to be a wound-healing response that is much more reversible [2, 3]. Percutaneous liver biopsy has long remained as the gold standard for diagnosis and staging hepatic fibrosis. However, its utility is limited by the invasiveness of the procedure, likelihood of sampling errors and inter-observer variations [4, 5].Therefore, noninvasive methods with sufficient sensitivity to identify liver fibrosis at early stage and monitor fibrosis progression and regression are of great clinical and therapeutic values. Diffusion time dependency of measured diffusion coefficient, being the evidence of restricted diffusion, has been widely observed in brain tissues [6, 7]. In liver fibrosis, the structural damage on cellular scale may contribute to a modification of the restricted diffusion behavior of water molecules in the intra- and extracellular space. In this study, we examined whether different diffusion times would yield different sensitivities in detecting the pathological alterations in tissue microstructure during liver fibrogenesis in an experimental rat model.

METHODS: ***Animal preparation***: Sixteen male adult SD rats (220–260g, 6 weeks old) were assigned to two groups. The fibrotic group (N=12) received subcutaneous injection of 1:1 volume mixture of carbon tetrachloride (CCl4) in olive oil at a dose of 0.2 mL / 100 g of body weight twice a week for 8 weeks [8]. The control group (N=4) received no injection. DW MRI was performed in animals 1 day before, 2, 4, 6 and 8 weeks after CCl4 administration. ***MRI***: All MRI experiments were performed using a Bruker 7T scanner. DW images (DWIs) with 5 different b-values (0, 200, 400, 800 and 1000 s/mm^2) along phase encoding direction (R-L) were acquired in one axial slice covering the liver with respiration-gated single shot stimulated-echo-EPI sequence. DW experiments were repeated with diffusion time Δ= 15, 45 and 200ms. Imaging parameters were TR/TE=~2000/20ms, δ=3.1ms, slice thickness= 3mm, FOV=51x51mm^2, acquisition matrix=51x51, NEX=11. ***Data analysis***: A large ROI excluding large blood vessels was drawn on liver parenchyma encompassing a large homogeneous liver region. Apparent diffusion coefficient (ADC) was obtained by fitting the equation: $SI_b/SI_0=\exp(-b\times ADC)$ with the ROI measurements of SI/SI_0 at all five b values (0, 200, 400, 800, 1000 s/mm^2) using a least-square nonlinear fitting in Matlab. True diffusion coefficient (D_{True}) was estimated by fitting the signal decay in the ROI on the images of b values higher than 200 s/mm^2 (400, 800 and 1000 s/mm^2) to the equation: $SI_b/SI_0=(1-f)\times\exp(-b\times D_{True})$. One-way ANOVA with Turkey's multiple comparison tests was employed to compare ADC, and D_{True} measurements between different time points of liver fibrosis, as well as different Δ, and $p< 0.05$ was considered as statistical significant.

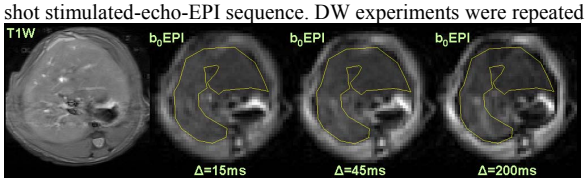

Fig. 1 Representative T1W and B0 EPI images with diffusion time Δ=15,45 and 200ms from one adult SD animal after 8-week CCl4 insult.

RESULTS: Fig.1 shows the representative T1W and B0 EPI images of Δ=15,45and 200ms from one fibrotic animal. Typical ROI used for ADC and D_{True} measurement is also illustrated. Fig. 2 shows that the mean DW liver signal decay, computed as the average of SI_b/SI_0 from 6 animals after 8-week CCl4 insult, as a function of b-values at different times (Δs). Fig. 3 shows the liver ADC and D_{True} values measured with Δ = 15, 45, 200 ms at different time points of liver fibrosis. ADC, D_{True} generally decreased with Δ in both normal and fibrotic liver, confirming their diffusion time dependency and spatially restricted diffusion in liver. Fig.4 compares the liver ADC and D_{True} values measured at five different stages of liver fibrosis for three different times. The comparisons demonstrate that D_{True} yielded higher sensitivity than ADC in detecting cellular changes during liver fibrosis. Specifically, greater statistical significances were generally observed when comparing D_{True} between 0-week, 2-week and 4-week CCl_4 insult at three different Δ (Fig. 4 right). Moreover, the percentage changes of D_{True} from week2 to week4 after CCL4 insult were greater at Δ = 200ms (15±3.3%) than Δ = 45ms (12±4.1%) and Δ = 15ms (9±3.2%), indicating that long diffusion time improved D_{True} sensitivity. ADC and D_{True} of liver from age-matched control group exhibited no significant differences over different time points from liver fibrosis group at all Δs (not shown) as expected, confirming the robustness of the DW and analysis protocol employed in the current study. Note that the diffusion distance corresponding to diffusion time of Δ= 15~200ms ranges approximately from 5μm to16μm in normal liver as estimated using Einstein's equation. Also note that our histological results confirmed that the livers were at different stages of fibrosis during different periods after CCl4 administration (not shown) as expected.

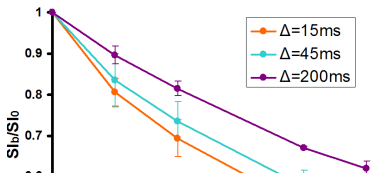

Fig. 2 Typical mean DW signal decays, computed as the average of SI_b/SI_0 from 6 fibrotic animals, for different diffusion times (Δs).

DISCUSSION AND CONCLUSION: First, the decrease in ADC and D_{True} observed after CCL4 insult (2-week to 8-week) likely resulted from the excessive extracellular matrix (ECM) especially collagen fiber deposition, one of four central features in the process of lipocyte activation induced by chronic liver injury [2, 9], leading to higher content of diffusion barriers in the extracellular space in the scar tissue of fibrotic liver. ADC and D_{True} decrease at early phase after CCL4 insult (week 2 to week 4) might also be related to the proliferation of hepatic lipocyte which is another feature during lipocyte activation [2, 9]. Cell proliferation causes an increase in cell density and cell membranes. Cell membranes hinders and highly restricts the water diffusion process inside the cells [10]. In addition, high cell density results in intracellular water fraction increase and extracellular water fraction decrease in tissue, leading to more restricted water diffusion and lower D_{true} and ADC. In fact, it is widely believed that diffusion decreases in the tissue undergoing cell proliferation [11, 12]. Secondly, the mean D_{true} reductions between Δ =15 and Δ =45 ms, 45 ms and 200 ms, 15 ms and 200 ms in liver after 2-week CCl4 insult were 8±2.2%, 14±3.4% and 21±4.1%, respectively; while those in liver after 4-week CCl4 insult were 10±3.2%, 18±3.8% and 26±6.4%, respectively (Fig.3). The substantial decreases in D_{True} with Δ at week 4 might be due to that more barriers, such as increased cell membranes and accumulated collagen fibers, were encountered by water molecules diffusing in intra-and extracellular space in fibrotic liver. Note that ADC and D_{True} at different stages of fibrosis varied with Δ to different extents, suggesting that it may be desirable to use two different Δs to detect cellular changes caused by fibrosis. Finally, liver ADC and D_{True} of age-matched control group showed no significant differences over different time points of liver fibrosis at all Δs, confirming that the changes in ADC and D_{True} were caused by fibrosis rather than liver maturation. To conclude, true diffusion coefficient (D_{True}) measured with long diffusion time is highly sensitive in detecting and accessing subtle changes in tissue microstructure during liver fibrogenesis.

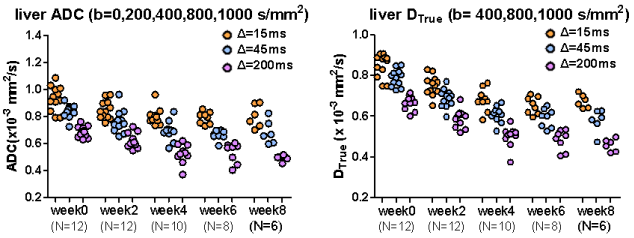

Fig. 3 ROI measurements of liver ADC and D_{True} obtained from DWI with Δ = 15, 45, 200 ms at different time points of liver fibrosis.

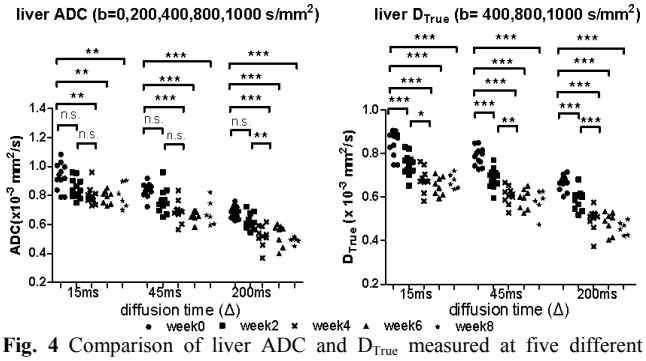

Fig. 4 Comparison of liver ADC and D_{True} measured at five different stages of liver fibrosis with three diffusion times using one-way ANOVA with * for $p<0.05$, ** for $p<0.01$, *** for $P< 0.001$.

REFERENCES: **1.** Friedman SL, JHEPAT (2003). **2.** Friedman SL et al., NEJM (1993). **3.** Albanis E et al., Clinics in Liver Disease, 2001. **4.** Abdi W et al., Arch Intern Med (1979). **5.** Regev A et al., AJG (2002). **6.** Horsfield MA et al., MRM (1994). **7.** Beaulieu C et al., MRM (1996). **8.** Constandinou et al., Methods Mol Med (2005). **9.** Bataller R. JCI (2005). **10.** Szafer A et al., MRM (1995). **11.** Togao O et al., Radiology (2010). **12.** Lyng H et al., MRM (2000).