Intra-Voxel Incoherent Motion MRI in Rodent Model of Diethylnitrosamine-Induced Liver Fibrosis

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Introduction

Intra-voxel incoherent motion (IVIM) imaging permits *in vivo* quantification of the microscopic translational motion of water that occurs due to molecular diffusion and/or microcirculation of blood within capillary networks (perfusion) [1, 2]. Conventional apparent diffusion coefficient (ADC) measurements integrate the contributions of both diffusion and perfusion components [1, 3]. Hepatic fibrosis significantly alters liver perfusion. During previous studies in the CCl_4 rodent model [4], decreases in liver ADC levels were well correlated to increased liver fibrosis. The latter relationship was attributed to altered perfusion levels [1]. Recent clinical studies suggest that IVIM approaches offer the potential to differentiate the contributions of perfusion and water mobility during diffusion-weighted (DW) signal decay measurements in the liver [5, 6]. The purpose of our study was to investigate the relationship between hepatic IVIM measurements and diethylnitrosamine-induced fibrosis levels in the Wistar rat model. Our hypothesis was that IVIM perfusion fraction measurements would be negatively correlated to increasing hepatic fibrosis levels.

Materials and Methods

Animal Model Adult male Wistar rats (n = 17, weight 350 – 400g) were used for our ACUC-approved experiments. Liver fibrosis was induced in thirteen rats by oral gavage once a week with 5mL/kg dose 1.5% DEN solution (DEN ISOPAC®, Sigma Chemical, USA). 4 untreated rats were used as controls.

MR Imaging All experiments were performed using a 3.0T clinical MR scanner (Magnetom Trio, Siemens Medical Solutions) with custom-built rodent receiver coil (Chenguang Medical Technologies Co., Shanghai, China). Imaging was performed for two rats on weeks 4, 5, 6, 7, for one rat on weeks 9 and 10 and for three rats on week 8 of DEN administration. Prior to imaging, rats were anesthetized with an intra-muscular injection of ketamine (80mg/mL) and xylazine (10mg/mL). The abdomen of each rat was fixed with a belt of adhesive tape to limit respiratory motion. Coronal and transverse T2-weighted TSE images of the entire liver were acquired for localization. Axial IVIM scans were performed using a diffusion-weighted EPI sequence with the following imaging parameters: TR/TE=3500/71ms, 1260kHz/pixel BW, 5/8 partial Fourier, EPI factor = 52, 4 signal averages, 3mm slice thickness, 150×61 mm^2 FOV, 52×128 matrix (1.2 x 1.2 x 3 mm^3 voxel size), and fat-sat preparation. Imaging was performed during free-breathing with respiratory belt triggering at expiration. DW images were acquired with gradient factors b = 0, 50, 100, 150, 200, and 300 sec/mm^2.

Image Analysis Data analysis was performed offline using MATLAB software. Based on IVIM theory, DW signal attenuation is described by the following equation [1, 3]: $SI/SI_0=(1-f)\times exp(-bD)+f\times exp(-bD^*)$, where D and D^* are the true diffusion coefficient and the pseudo-diffusion coefficients, respectively, and f is the perfusion fraction(fractional volume of the voxel occupied by the flowing spins). For each imaging slice, a conventional ADC map (ADC_{conv}) was first generated on a voxel-by-voxel basis: $ADC_{conv}=\mathrm{Log}(S_1/S_5)/(b_5-b_1)$, where b_1=0, b_5=200 sec/mm^2. When the DW gradient strengths are sufficiently strong, ADC measurements begin to approximate the true diffusion coefficient D. Hence for each imaging slice, a diffusion map (D) was generated on a voxel-by-voxel basis: $D=\mathrm{Log}(S_3/S_6)/(b_6-b_3)$, where b_3=100, b_6=300 sec/mm^2. Next, using these DW images at b=0, 50, and 300 sec/mm^2, perfusion fraction (f) was estimated according to methods of Le Bihan et al [1]. ROIs within liver parenchyma were drawn for each slice on b=0 sec/mm^2 images (avoiding blood vessels) and these ROI were transferred to the corresponding IVIM functional maps for mean ADC_{conv} and D measurements in each animal.

Histological Analysis Following each imaging study, animals were euthanized and livers harvested for histological evaluation. Liver specimens were fixed in formalin and paraffin embedded. Masson's tri-chrome staining was used to identify collagen tissues. Histological slides were digitized with x10 optical magnification using a multi-channel image acquisition system (TissueGnostics, Vienna, Austria). Specimens were evaluated by an experienced GI pathologist and a quantitative assessment of liver fibrosis was performed to describe the total percentage fibrotic parenchymal area [7].

Statistical Analysis All statistics were performed using SPSS (SPSS, Chicago, IL, USA). The Spearman's correlation coefficient was calculated to assess the correlation between the percent of liver fibrosis and ADC_{conv}, D, and f levels. Test was considered statistically significant with a p-value < 0.05.

Results

Histological liver specimens from the healthy control and the DEN-induced fibrosis rats were strikingly different on microscopic examination (**Fig. 1**). The values for perfusion fraction f, ADC_{conv} and D are plotted against percent fibrosis level in **Fig. 2**. Both ADC_{conv} (**Fig. 2a**) and perfusion fraction f (**Fig. 2c**) measurements were well correlated (negatively) with the fibrosis levels (ADC_{conv}: r=-0.8145, p<0.001; f: r=-0.9343, p<0.001), while D (**Fig. 2b**) was poorly correlated(r=-0.5408, P=0.0250). Representative ADC_{conv} and D maps for three animals with different fibrosis levels are shown in **Fig. 3**.

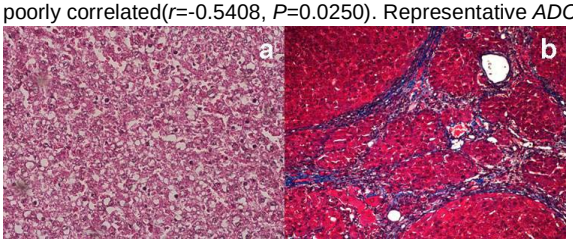


Fig 1. (Left) Tri-chrome histological slides of rat liver tissues. Normal rats showed no fibrosis (**a**) whereas DEN rats (**b**) demonstrated significant levels of fibrotic tissue.

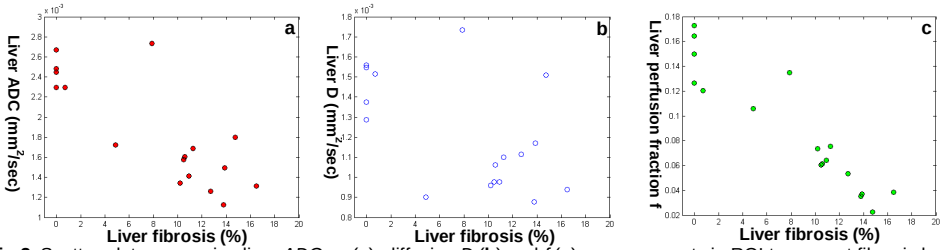
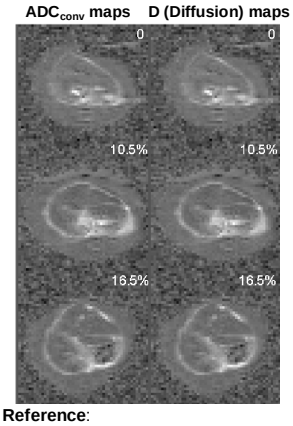


Fig 2. Scatter-plots comparing liver ADC_{conv} (**a**), diffusion D (**b**) and f (**c**) measurements in ROI to percent fibrosis levels in 17 rats. A significant negative correlation observed for f (r=-0.8735, p<0.001) and ADC_{conv} (r=-0.9343, p<0.001).

Fig 3. (Right) Axial ADC_{conv} and D (diffusion) maps of Wistar rat livers at different fibrosis levels. Note: %fibrosis level listed in the upper corner of each image.



Conclusions

Decreasing perfusion fraction f with increasing liver fibrosis suggests that the observed corresponding ADC decreases are due to blood flow alterations with progressive disease. Further studies are necessary to fully characterize the sensitivity and specificity of IVIM methods for staging disease progression. Nonetheless, our current results in the diethylnitrosamine-treated rodent model suggest that these IVIM approaches offer a promising non-invasive method for the diagnosis of liver fibrosis and functional assessment hepatic perfusion.

Reference:

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