

Intravoxel Incoherent Motion Diffusion-weighted Imaging in Liver Fibrosis of Rat with Bile Duct Ligation

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INTRODUCTION: Intravoxel Incoherent Motion (IVIM) Diffusion-weighted magnetic resonance Imaging (DW-MRI) is a sensitive method to visualize the molecular Brownian motion of water (true diffusion) and the microcirculation of blood (pseudodiffusion or perfusion-related diffusion) in biologic tissue [1, 2]. The true diffusion coefficient (D), blood pseudodiffusion coefficient (D*), perfusion fraction (f), determined from DW-MRI on the basis of the IVIM theory have been investigated in human liver cirrhosis and animal models [3-5]. We previously reported that, there was a reduction in Dtrue (D) and Dpseudo (D*) values in rats liver fibrosis with carbon tetrachloride injection [5]. Experimental biliary obstruction by ligation of the common bile duct has been widely used as a liver fibrosis animal model [6]. This study aims to further investigate the alteration of D, D* and f in the rat liver fibrosis induced by bile duct ligation.

MATERIALS AND METHODS: Animal Preparation:

There were 8 normal male Sprague-Dawley rats (200 ± 20 g) were used in this study. DW-MRI was performed before the bile duct ligation operation (D0), on the 3rd day (D3) and 7th day (D7) after the surgery. All rats were anesthetized with 10% chloral hydrate during MR scan. **MRI Protocol:** All MRI measurements were acquired utilizing the Siemens Avanto 1.5 T clinic scanner with a phased array coil for rat (Shanghai Chenguang Medical. Technologies Co., Ltd). T1W and T2W were acquired using turbo spin echo, repetition time (TR) / echo time (TE) = 1700/20ms for T2W and TR/TE = 500/10ms for T1W, field of view (FOV) = 150 x 112mm². DW imaging was performed in axial slices covering the liver using single-shot spin-echo EPI (SE-EPI) with 12 b-values (0, 50, 100, 150, 200, 250, 300, 400, 500, 650, 800 and 1000 sec/mm²) and three diffusion gradient directions, TR/TE = 2000/83ms, FOV = 183 x 183 mm², acquisition matrix = 128 x 128, slice thickness = 3mm and number of averages (NEX) = 5. **Data Analysis:** All DW images were analyzed on the Siemens MR Workstation and fitting in MatLab. A region of interest (ROI, large than 0.25 cm², 12 pixels) was defined to measure signal intensity (SI) at all b values in the right lobe of liver to minimize the cardiac and respiratory motion effects. The same ROI was then used to measure the apparent diffusion coefficient (ADC) on ADC map generated on the Workstation. Care was taken to maintain ROIs of similar size and location in different animals at different time points. The true diffusion, D was estimated by using only b values greater than 200 sec/mm², with a simple linear fit equation: $SI_b/SI_0 = \exp(-b \times D)$ [3]. D* and f were estimated by fitting with the IVIM bi-compartmental model: $SI_b/SI_0 = (1-f) \times \exp(-b \times D) + f \times \exp(-b \times D^*)$ with a least-square nonlinear fitting in MatLab. All data were expressed as mean ± SD. The D, D*, f and ADC in rat livers on D0, D3 and D7 were compared with One-way ANOVA test. P < 0.05 was considered as statistical significant. **Histology Preparation:** After the D7 MRI experiments, 3 rats were selected for histological HE staining to observe liver injury.

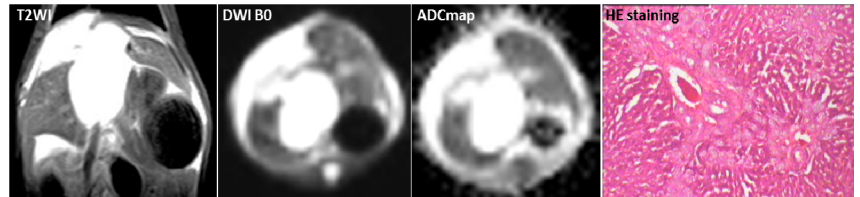


Fig.1 T2WI, DWI at b=0, ADC map and HE staining (magnification x 100) in a rat model.

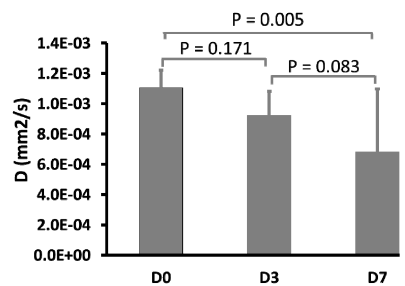


Fig.2 D in rat livers on D0, D3 and D7.

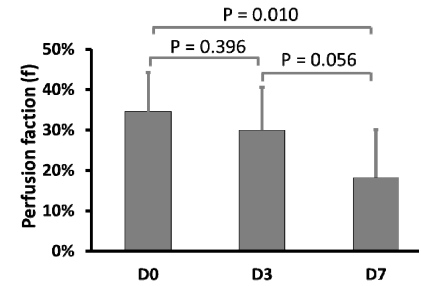


Fig.3 f in rat livers on D0, D3 and D7.

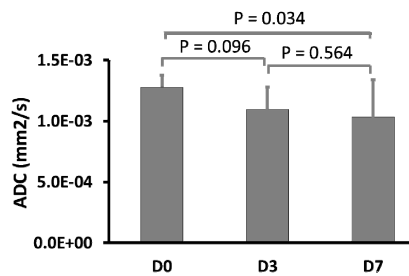


Fig.4 ADC in rat livers on D0, D3 and D7.

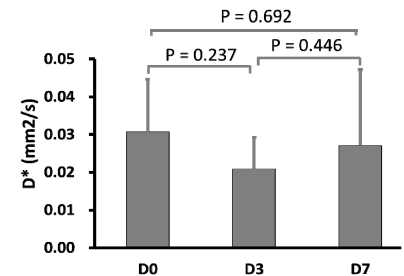


Fig.5 D* in rat livers on D0, D3 and D7.

RESULTS: Fig.1 showed a typical T2WI, DWI at b=0, ADC map and HE staining slice in a rat model. Fig.2-4 showed a decreasing trend of D, perfusion fraction (f) and ADC of the livers from D0 to D3, D7. And the differences in D, f, and ADC approached statistical significance on D7 compared to D0 (D: 0.680 × 10⁻³ mm²/s ± 0.417 v.s. 1.10 × 10⁻³ mm²/s ± 0.116; f: 18.2 % ± 11.9 v.s. 34.6 % ± 9.73; ADC: 1.03 × 10⁻³ mm²/s ± 0.308 v.s. 1.28 × 10⁻³ mm²/s ± 0.099). Fig.5 showed no difference in D* of the livers from D0 to D3, D7. The HE staining showed the liver injury induced by bile duct ligation, including periportal inflammation and fibrosis, bridging necrosis, and an extended extracellular space in the liver.

DISCUSSIONS: The results of this study suggested, during the progression of liver fibrosis in the bile duct ligation model, there was a reduction in D, f and ADC values possibly resulted from diffusion and perfusion changes, respectively. Bile duct ligation induces an acute obstructive jaundice, and liver fibrosis is subsequently achieved between seven days to four weeks, and progression to cirrhosis in 4 or 6 weeks [7-8]. Liver fibrosis is characterized by an accumulation of extracellular matrix in the periportal space. The periportal fibrosis extensively alters the vasculature, affects the liver hemodynamic flow and depresses liver function [9]. Our results implicated that there was an alteration of hepatic microcirculation in the early stage of liver fibrosis induced by bile duct ligation. It was reported that there was minimal collagen fibers in liver on the 8th day after the surgery [10]. We suggested that periportal inflammation and marginal bile duct proliferation might contribute to the reduction of true diffusion. The variation of ADC in animal livers on D7 was possibly attributed to the heterogeneity of fibrosis, bridging necrosis and extended extracellular space observed in the liver histopathology. We also observed varied D* on D3 and D7 after the surgery. The large variation of D* was partly due to the estimation by fitting DW signal decay in the ROIs with the IVIM bi-compartmental model [1-2].

CONCLUSION: The reduction of true diffusion and perfusion fraction were observed in the early stage of rat liver fibrosis induced by bile duct ligation. The heterogeneity of fibrosis, bridging necrosis and extended extracellular space would contribute to the variation of apparent diffusion coefficient during the progression of liver fibrosis.

REFERENCES: 1. Lebihan D, et al. Radiology 1986; 161(2): 401-407. 2. Lebihan D, et al. Radiology 1988; 168(2): 497-505. 3. Luciani A, et al. Radiology. 2008; 249(3): 891-9. 4. Lee JT, et al. Magn Reson Imaging. 2012; 30(4): 572-8. 5. Chow AM, et al. J Magn Reson Imaging. 2012; 36(1): 159-67. 6. Kountouras J, et al. Br J Exp Pathol. 1984; 65: 305-311. 7. Ronald J. Sokol, et al. Semin Liver Dis 2001; 21(4): 517-524. 8. Hernandez-Gea V, et al. Annu Rev Pathol. 2011; 425-456. 9. Marques TG, et al. Acta Cir Bras. 2012; 27(8): 589-94. 10. Wang YX, et al. Radiology. 2011; 259(3): 712-9.