In Vivo Assessment of Hepatic Ischemia/Reperfusion Injury in Rat Using Diffusion Tensor Imaging

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Introduction

Hepatic ischemia/reperfusion injury (IRI) induces cellular damage. Hepatic IRI occurs during liver transplantation, tumor resection, hemorrhagic shock and veno-occlusive disease, and is a major cause of acute liver failure that is associated with high morbidity and mortality [1]. IRI in liver is also responsible for early organ failure and increased incidence of both acute and chronic rejection after liver transplantation [2]. A noninvasive indicator is desired to detect and assess liver IRI in such circumstances. The diffusion of water molecules in biologic tissue due to random thermal motion is widely used to characterize various normal or disease states in central nervous system. Since hepatic edema occurs immediately after hepatic IRI [3], we hypothesize that diffusion properties in liver would change and therefore could be probed by diffusion tensor imaging (DTI). In this study, we aim to investigate whether DTI can be used to monitor hepatic IRI in Sprague-Dawley (SD) rats longitudinally.

Methods

Animal Procedures: The employed rodent model of total hepatic IRI was performed as described previously in rats [4]. Six normal SD rats (220-250 g) were anesthetized with an intraperitoneal (i.p.) injection of 100 mg/kg ketamine, and 10 mg/kg xylazine. The abdomen was shaved and a midline incision was made. The common portal vein, hepatic artery and bile duct in the hepatoduodenal ligament were clamped using a vascular clamp. The liver was inspected for ischemia for 2 minutes. After 30 minutes of hepatic ischemia, the clamp was removed initiating hepatic reperfusion. The liver was again inspected for restoration of blood flow, then abdomen was closed and the animal was kept at ambient temperature of 37°C. Animals were scanned with DTI at 1 day before injury, 120 minutes and 1 day after IRI.

MRI: All MRI experiments were performed on a 7 T Bruker MRI scanner with a 60 mm quadrature RF resonator. Animals were fasted before scanning to reduce peristalsis. Each rat was anesthetized with isoflurane/air using 1.0-1.5 % for maintenance. Body temperature was maintained at about 36.5°C by circulating warm water in a heating pad with respiratory monitoring. Diffusion tensor imaging (DTI) was performed on one axial slice passing through the liver with respiratory-gated single-shot spin-echo echo-planar imaging (SE-EPI) sequence using TR ≈ 2.5 s, TE = 33 ms, FA = 90º, two b-values were used (0 and 1000 s/mm^2) for 6 diffusion gradient directions, FOV = 5.12×5.12 cm, slice thickness = 2 mm, acquisition matrix = 64×64, voxel size = 0.8×0.8×2 mm^3, NEX = 10, and total scan time of ~3 min. Note that high b value (1000 s/mm^2) was used to decrease the influence of perfusion effect in diffusion measurement [5]. Diffusion weighted (DW) images were first co-registered using AIR5.2.5 [6]. Mean diffusivity (MD) and fractional anisotropy (FA) maps were generated using DTIStudio [7]. A large region of interest (ROI) was drawn in homogeneous region of liver parenchyma for MD and FA measurements. Analysis of variance (ANOVA) test was employed to compare the differences between different time points of hepatic IRI. P values less than 0.05 were considered statistically significant. In order to examine the individual effects of molecular diffusion of water and microcirculation of blood on MD changes, three of the animals were also scanned with DW imaging with 7 b-values (0, 200, 400, 800, 1200, 1600, and 2000 s/mm^2) and single direction, TE = 40 ms, and all other parameters were the same as the DTI sequence above. Diffusion coefficient (D) and perfusion fraction (f) were estimated by fitting DW signals to a bi-compartment model using $SI/SI_0 = (1 - f) \times \exp(-b\ D) + f \times \exp(-b\ D^*)$, where D* is the blood pseudodiffusion coefficient [5].

Histology: One animal was sacrificed on the day of injury after MRI and another animal was sacrificed one day after injury following MRI. The livers were perfused transcardially and embedded in paraffin. The livers were then sectioned and examined by light microscopy after standard hematoxylin-eosin (H&E) staining.

Results and Discussion

Fig. 1a, 1b and 1c show the typical MD maps of a rat liver at 1 day before, 120 minutes and 1 day after hepatic IRI respectively. Fig. 2a and 2b show the MD values and FA values versus time (N = 6) respectively. The MD values at 120 minutes after IRI ($0.75 \pm 0.06 \times 10^{-3}$ mm^2/s) were significantly lower ($P < 0.05$) than those at 1 day before injury ($1.01 \pm 0.04 \times 10^{-3}$ mm^2/s) and 1 day after injury ($1.03 \pm 0.05 \times 10^{-3}$ mm^2/s) while FA values after IRI (0.33 ± 0.03) were significantly higher ($P < 0.05$) than those at 1 day before injury (0.21 ± 0.02) and 1 day after injury (0.20 ± 0.02). The MD and FA differences between 1 day before injury and 1 day after injury were not statistically significant. Fig. 3 demonstrates the non-monoexponential decay of DW signal typically observed in liver, with the blood perfusion manifesting the fast pseudodiffusion effect. Fig.4a and 4b show the D values and perfusion fraction f versus time (N = 3) respectively, indicating that both D and perfusion fraction decreased at 120 minutes after injury. As in cerebral ischemia injury, the MD/D decrease after hepatic IRI could arise from the decreased extracellular water caused by cytotoxic edema after injury [3] as shown in Fig. 5a. Decrease in perfusion fraction could be due to the disturbances of microcirculation caused by sinusoidal congestion and blood cell-endothelial cell adhesion [1]. FA increased likely because the anisotropic nature of radially oriented structures in hepatic plates became more obvious due to increased intracellular water. MD/D and FA then normalized afterwards suggesting that intracellular edema in the ischemic lobes was being resolved gradually [3] and indicating cell necrosis/apoptosis as shown in Fig. 5b.

Conclusion

The experimental results demonstrated that DTI is useful in identifying hepatic IRI by characterizing the transient changes in diffusion parameters. DTI may also be potentially applicable in evaluation of drug pretreatment and ischemic preconditioning in hepatic IRI.

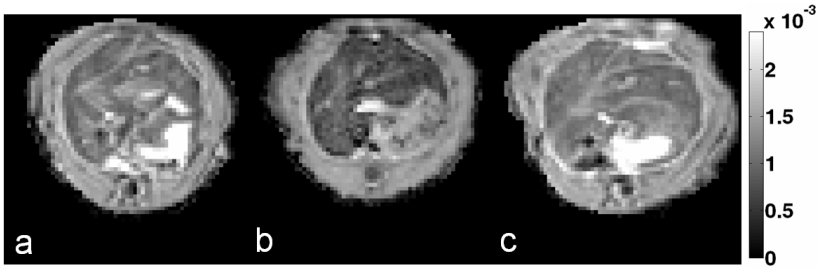

Fig. 1. Typical MD maps: (**a**) 1 day before injury; (**b**) 2 hrs after hepatic IRI; (**c**) 1 day after injury. Three maps are displayed in the same scale (in mm^2/s).

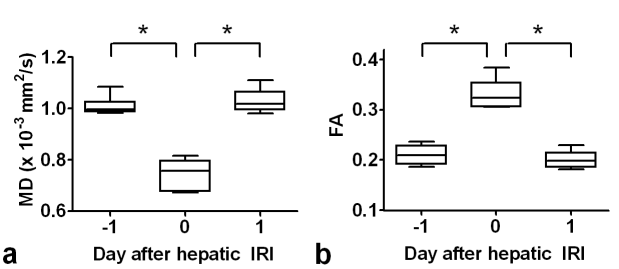

Fig. 2. (**a**) MD and (**b**) FA values at different time points (N = 6): 1 day before, 2 hrs after and 1 day after hepatic IRI. * $P < 0.05$.

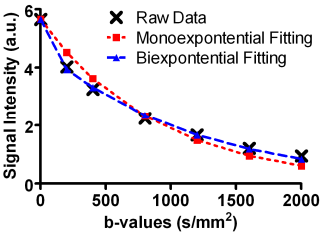

Fig. 3. DW signal intensity versus b-values demonstrating non-monoexponential decay.

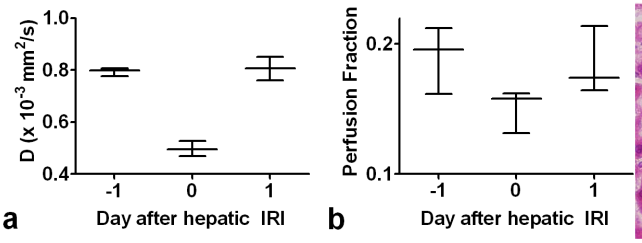

Fig. 4. (**a**) D and (**b**) perfusion fraction f values during the course of hepatic IRI (N = 3) estimated using a bi-compartment diffusion model.

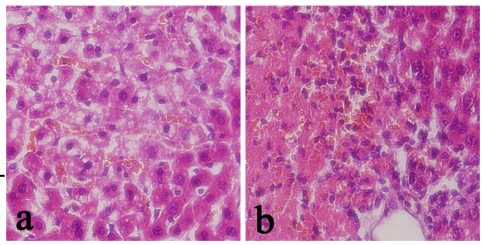

Fig. 5. Liver histological changes at (**a**) ~2 hrs after hepatic IRI and (**b**) 1 day after injury.

References [**1**] GF Douglas et al, Transtant Rev 2000;14:106-126. [**2**] JW Kupiec-Weglinski et al, Transplant Proc 2005;37:1653-1656. [**3**] AB Lentsch et al, Hepatology 1998;27:1172-1177. [**4**] T Nishida et al, Am J Physiol Heart Circ Physiol 2000;278:H1565-1570. [**5**] I Yamada et al, Radiology 1999;210:617-623. [**6**] RP Woods et al, J Comput Assist Tomogr 1998;22:139-152. [**7**] H Jiang, et al. Comput Methods Programs Biomed 2006;81:106-116.