Carbogen Gas-Challenge BOLD MRI in Rat Liver Fibrosis Model

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INTRODUCTION

Hepatic fibrosis is a dynamic and reversible process (1); hence early diagnosis is critical. In the normal liver, approximately three quarters of the blood supply is from the portal vein and only about one quarter originates from the hepatic artery. Disease progression leads to both regional and global alterations to hepatic perfusion levels (2). Portal venous flow decreases whereas hepatic arterial flow increases (3). Previous BOLD studies in normal rats during hyperoxia (induced via carbogen inhalation) demonstrated that T2*-weighted signal intensity changes were primarily due to the reduced deoxyhemoglobin concentration levels in the portal venous system (4). For the current study, our hypothesis was that in the setting of liver fibrosis with progressive decreases in portal blood supply, BOLD signal changes between normal and hyperoxia conditions would be reduced. The purpose of study was to investigate the relationship between gas-challenge BOLD response and the degree of liver fibrosis.

METHOD

Animal Model Adult male Wistar rats (n = 21, weight 350 – 400g) were used for our ACUC-approved experiments. Liver fibrosis was induced in eighteen rats by weekly oral gavage with 5mL/kg dose 1.5% DEN solution (DEN ISOPAC®, Sigma Chemical, USA). 3 untreated rats were used as controls.

MRI Before imaging, rats were anaesthetized with high limb injection of ketamine (80mg/kg) and xylazine (10mg/kg). Imaging was performed for two rats on weeks 4, 5, 6, 7, 9 10 and for three rats on weeks 8 and 11 of DEN administration. We used a 3T clinical scanner (Magnetom Trio, Siemens) with custom-built rodent receiver coil (Chenguang Med. Tech. Co., Shanghai, China). Coronal and transverse T2-weighted TSE images of the entire liver were acquired for localization. Three representative axial slices passing through the central part of the liver were selected for our BOLD studies. For R2* measurements we used a multi-gradient-echo (MGRE) sequence with following parameters: TR=150ms, ETL=12 (4.6ms spacing), FA= 30°, 3 mm slices, 150mm FOV, 192 matrix, averages = 25. Room air (78% N_2/20% O_2) or carbogen (95%O_2/5%CO_2) was administered via a nose-cone. MGRE images were first acquired during air breathing; then the animal was given carbogen for ten minutes for transition, and a second set of MGRE images were acquired while the animal continued to breathe carbogen. Next, a dynamic time series of MGRE images were acquired for 60 mins (90 scans) during gas challenge at 10 mins intervals: carbogen – air – carbogen – air – carbogen – air (4 signal averages for each R2* measurement). Following each imaging study, animals were euthanized and livers harvested for histological evaluation.

Images Analysis R2* maps during each stage of gas inhalation were calculated by employing the non-linear Levenberg-Marquardt algorithm to fit the mono-exponential function $S(TE_i) = S(0)\cdot\exp(-R2^*\cdot TE_i)$ using Matlab software (The Math Works Inc., Natick, MA). Gas-challenge R2* change maps were calculated as R2* air – R2* carbogen. For each animal a region of interest (ROI) was drawn in the liver parenchyma, excluding blood vessels, to measure mean R2* change values.

Histology Liver specimens were fixed in formalin and paraffin embedded. Masson's trichrome staining was used to identify collagen tissue. Specimens were evaluated by an experienced pathologist according to METAVIR scoring system on a 0-4 scale (5) and a quantitative assessment of liver fibrosis was performed to describe the total percentage fibrotic parenchymal area (6).

Statistical Analysis All statistics were performed using SPSS (SPSS, Chicago, IL, USA). One-way analysis of variance (ANOVA) was used to compare the percentage of fibrosis and the mean R2* changes in three groups, Fibrosis stage 0 (F0), stage 1-3 (F1-3) and stage 4 (F4). Spearman's correlation coefficient was used to assess the correlation between the percentage of liver fibrosis and gas-challenge R2* changes. Tests were considered statistically significant with a p-value < 0.05.

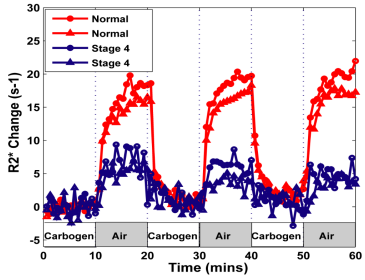


Fig. 1 Dynamic hepatic R2* changes in response to repeated carbogen gas challenges in 2 normal rats and 2 stage 4 fibrosis rats.

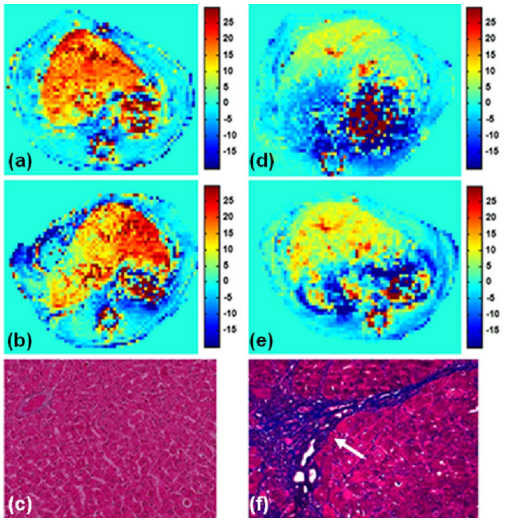


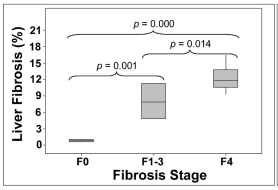
Fig. 2 Gas-challenge R2* changes decreased with increasing level of fibrosis (representative ΔR2* maps for normal: **a** and **b**; stage 4 fibrosis: **d** and **e**). Histological sections of rat livers showed no fibrosis in normal rats (**c**), whereas severe fibrosis (arrow) was seen in stage 4 animals (**f**).

RESULTS

R2* increases and decreases were highly reproducible and well correlated to the gas challenge intervals during the dynamic BOLD studies (**Fig. 1**). R2* changes between air and carbogen breathing for normal rats were clearly much greater than R2* changes measured in fibrotic animals (**Fig. 1** and **Fig. 2a, b, d, and e**). Pathology specimens demonstrated striking alterations to the normal liver parenchyma with widespread collagen deposition throughout the organ (**Fig. 2c and f**). We found statistically significant differences among all three groups when comparing the percentage of fibrosis and R2* changes. The percentage of fibrosis increased with increasing METAVIR stage (F0: 0.89% ± 0.22%; F1-3: 8.03% ± 3.20%; F4: 12.14% ± 2.03%) (**Fig. 3a**). Compared to Stage 4, R2* changes were significantly greater in Stage 0 and Stage 1-3 (F0: 20.3 ± 6.0 s^{-1}; F1-3: 16.2 ± 5.2s^{-1}; F4: 5.4 ± 6.8 s^{-1}) (**Fig. 3b**). There was a significant negative correlation between gas-challenge R2* changes and the percentage of liver fibrosis (r = -0.764, p = 0.0001) (**Fig. 4**).

CONCLUSION

Liver fibrosis is the response to chronic liver injury and can result in liver failure, cirrhosis and portal hypertension. During the progression of the disease, disruption of normal tissue structures leads to liver perfusion changes. In this work, a negative correlation was found between the BOLD response to gas-challenge and the degree of liver fibrosis. Gas-challenge BOLD MRI is a potential non-invasive method for liver fibrosis diagnosis and staging.



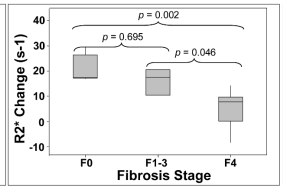


Fig. 3 Box plots demonstrating expected increase in the percentage of liver fibrosis with METAVIR stage (**a**) and decreasing gas-challenge R2* changes with increasing METAVIR stage (**b**).

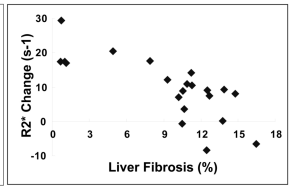


Fig. 4 Negative correlation was observed between the percentage of liver fibrosis and gas-challenge R2* changes (r = -0.764, p = 0.0001).

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