Assessment of Liver Fibrosis by Combined Measurements of T2 and Perfusion Parameters: A Preliminary Rat Study at 3.0 T

H. Kim[1], O. Haq[2], M. Qiu[1], M. R. Loureiro-Silva[2], P. M. Murphy[3], R. J. Groszmann[2], R. T. Constable[1]

[1]Diagnostic Radiology, Yale University, New Haven, CT, United States, [2]Hepatic Hemodynamic Lab, VACHS, Internal Medicine-Digestive Diseases, Yale University, New Haven, CT, United States, [3]Global Clinical Sciences, Pfizer, Sandwich, United Kingdom

INTRODUCTION

In cirrhosis liver fibrosis is the main structural abnormality causing the development of portal hypertension (PH) and this syndrome is the most common and lethal complication of cirrhosis. PH is involved in the development of esophageal varices, ascites and portal-systemic encephalopathy. The current gold-standard for assessing liver fibrosis and portal pressure (PP) remains biopsy and the measurement of the hepatic venous pressure gradient (a surrogate of PP measurement), respectively. The use of these techniques, particularly in clinical trials, is limited by their invasive nature. A reproducible non-invasive measure of fibrosis and liver hemodynamic abnormalities in cirrhotic patients would be a major improvement.

The purpose of this study was to explore the feasibility of MR applications to non-invasive assessment of liver fibrosis and PP by examining histological and hemodynamic changes in cirrhotic rats via estimation of T_2 and perfusion parameters and comparing these MR measures with liver histology and in vivo PP measurements.

METHODS

Induction of liver fibrosis in rats by CCl_4: Cirrhosis was induced in male Sprague-Dawley rats (final b.w.=398±85 g) by exposure to inhaled CCl_4 and addition of phenobarbital (0.35g/L) to drinking water [1]. Rats were used 6 to 10 days after the last dose of CCl_4 to avoid its acute inflammatory effect. Normal sex- and age-matched untreated rats were used as controls (b.w.=370±48 g).

MRI: Anesthetized (ketamine plus xylazine) rats were scanned on a 3.0 T Siemens Trio imaging system with a wrist coil (USA Instruments, Inc., Aurora, OH) for both transmission and reception. For T_2 measurements, a FSE sequence was used (TR=2500 ms, TE=8.2/84 ms, FOV=48.8 mm, 104×256 matrix, 10 averages, and 10 slices with a thickness of 3 mm). For the hepatic perfusion parameter measurements, an ultra-fast gradient echo sequence (VIBE, Siemens) was used (TR=2.84 ms, TE=1.47 ms, FOV=83.8 mm, 64×128 matrix, 1 average, 8 slices with a thickness of 5 mm, and a flip angle of 20°) to obtain time-dependent Gd-DTPA concentration curves (for ~ 3 min after Gd-DTPA administration with a temporal resolution of 1.4 sec) from hepatic artery (HA), portal vein (PV) and liver tissue. Representative images and concentration curves are shown in Figs.1 and 2, respectively.

Contrast agent: To avoid saturation effects and maximize dynamic range [2] a low dose (0.05 mM/Kg) of Gd-DTPA (OMNISCAN™ - Amersham Health Inc., Oslo, Norway) was injected via the tail vein, 10 sec after the onset of data acquisition.

Data analysis: For T_2 estimation, discrete ROIs were placed over central 3 slices, including as much liver tissue as possible while avoiding large blood vessels. For the liver perfusion study, a single compartment model was used to extract hepatic perfusion parameters from those concentration curves via a non-linear least-square fitting routine as previously described in ref. *2*, such as distribution volume (DV) and mean transit time (MTT) of Gd-DTPA, and arterial and portal blood inflow (ABF and PBF). Thus portal fraction (PF) and global hepatic blood inflow (HBF) were also obtained. The precision of the non-linear fitting was evaluated by defining a 'residual of fit' and only those results whose residual of fit was below a threshold were included in the final data analysis.

In vivo hemodynamic study: After MRI, rats were allowed to recover and had full access to food and water. The following day, rats were anesthetized (ketamine plus diazepam) and studied *in vivo* via measurement of PP through a strain-gauge transducers connected to an indwelling catheter placed in the superior mesenteric vein [3].

Histology: Following pressure measurements, rats were euthanized and liver tissue was collected for further histological analysis. After staining with Sirius Red (specific for fibrous tissue), fibrosis was quantified by using a Bioquant analyzer. Fibrosis was estimated by the ratio of the fibrosis area/total area and expressed in percentages (fibro%).

RESULTS

Results are expressed in mean±SD. PP in cirrhotic rats (n=10) was significantly ($p<0.002$) higher than in normal (n=5) rats (14.3±2.2 vs. 10.2±1.0 mmHg). Fibro% in cirrhotic livers was 29.7±1.6% (n=5) and negligible in normal livers.

T_2 of cirrhotic livers (n=10) was significantly higher (p=0.02) than that of normal (n=7) livers (44.3±4.4 vs. 39.5±2.6 ms) and was highly correlated with PP (r=0.70, p=0.02).

There were significant differences between the normal (n=4) and cirrhotic (n=4) rats in PF (94±5 vs. 76±8%; p=0.01), PBF (489±94 vs. 248±81 ml/min/100 ml liver tissue; p=0.01), and HBF (521±97 vs. 329±105 ml/min/100 ml liver tissue; p=0.04). However, differences in MTT (sec) and DV (%) between the two groups were not statistically significant.

DISCUSSION

Considering the large amount of tissue included in the ROIs and limited image resolution, the increased T_2 in correlation with PP in cirrhotic liver may suggest that alteration of micro-vascular density in the progression of the disease is dominant over other T_2-changing variables such as fat infiltration and deposition of collagen.

The observation of reduced PF, PBF and HBF in cirrhotic rats (81%, 51% and 63%, respectively, relative to that in normal rats) in this study is encouraging. However, the perfusion data was found to be highly sensitive to the performance of the perfusion model [2] and to the choice of the first-nonzero data points of the Gd-DTPA concentration curves as well as the limited SNR of the images. This explains the small number of cirrhotic rats included in the final data analysis.

In conclusion, these perfusion parameters, in combination with T_2, may potentially be useful indicators of altered hemodynamics and tissue characteristics in cirrhotic liver. The implications of higher T_2 in the cirrhotic rats need to be clarified with further studies. The performance of the perfusion model, particularly in assessing milder fibrosis, also requires further investigation before a final model can be determined.

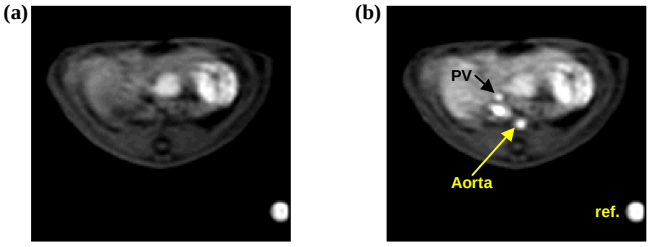

Fig. 1: MR images of the liver of a normal rat. (a): pre-contrast and (b): post-contrast (PV: portal vein, ref: reference phantom solution)

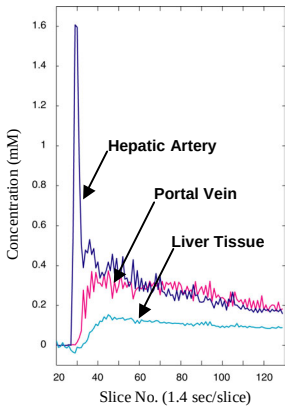

Fig. 2: The time-dependent concentration changes of Gd-DTPA in the portal vein, hepatic artery, and liver tissue

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