Hepatic Steatosis and Perfusion Parameters in the Progression of Liver Fibrosis: Findings in Carbon Tetrachloride (CCl4)-Treated Rats Using Three-Point Dixon and Dynamic Contrast-Enhanced MRI

H. Kim[1], C. J. Booth[2], A. B. Pinus[1], P. Chen[3], A. Lee[4], Y. Wang[3], M. Qiu[1], P. S. Murphy[5], and R. T. Constable[1,3]

[1]Diagnostic Radiology, Yale University, New Haven, CT, United States, [2]Section of Comparative Medicine, Yale University, [3]Biomedical Engineering, Yale University, [4]Biology, Yale University, [5]Clinical R&D, Pfizer, Sandwich, United Kingdom

INTRODUCTION

Liver fibrosis is a pathologic change caused by chronic liver damage and is characterized by deposition of excess amount of extracellular matrix (ECM) predominantly composed of collagen, which results in increased resistance to blood flow at the level of the sinusoids leading to portal hypertension and loss of portal blood perfusion [1]. Likewise, the presence of abnormally large amount of lipid within hepatocytes (steatosis) can also hinder liver perfusion by enlarging the liver cells giving rise to sinusoidal stenosis [2]. To our knowledge, however, altered hepatic perfusion in the progression of liver fibrosis has not been explored in association with hepatic fat content *in vivo*. Using 3-point Dixon (3PD) and dynamic contrast-enhanced (DCE) MRI this study explores the potential influence of hepatic fat content on MRI-detectable hepatic perfusion parameters in rats with carbon tetrachloride (CCl4)-induced hepatic fibrosis.

METHODS

Animal Preparation: Fibrosis was induced in 52 male Wistar rats by an intraperitoneal injection of CCl4 mixed with vegetable oil (25 µl CCl4 in a 150 µl volume (1:6)) 3 times per week for 2-16 weeks [3]. Six of the 14 control rats received pure vegetable oil at the same frequency. Prior to MRI scans rats were anesthetized (ketamine+xylazine) and an I.V. catheter was inserted into the tail vein of rats for administration of Gd-DTPA (OMNISCAN^{TM}-Amersham Health Inc., Oslo, Norway).

MRI: All images were collected on a 1.5 T Siemens scanner with a wrist coil (USA Instruments, Inc., Aurora, USA). For fat quantification, 3PD data were acquired using TrueFISP (Siemens; TR = 7.7 ms, TEs = 2.69/3.85/5.01 ms, FOV = 140 x 96, matrix size = 176 x 256, number of averages (NA) = 4, flip angle (α) = 55^o, 7-8 slices (4.5 mm thick)). For hepatic perfusion parameter estimation, a gradient echo sequence was used (TR/TE = 15/2.1 ms, FOV = 180 x 120, matrix size = 119 x 192, NA = 1, α = 40^o, 1 slice (3 mm thick)). Following a 15 sec. baseline scan, a bolus of Gd-DTPA (0.05 mM/kg) was administered and time-dependent signal changes in the aorta, the portal vein (PV) and liver tissue were continuously recorded as previously described [4,5] for approximately 4 min 30 sec (1.3 sec/image).

Data Analysis: The 3PD images were reconstructed from raw data according to the iterative least-squares estimation algorithm [6] written in MATLAB^{TM} (MathWorks Inc., Natick, USA). Fat-to-water-ratio (FWR) images were obtained from the calculated water-only and fat-only images, and mean FWRs over the imaged liver tissue were used as a measure of fat content. For the perfusion data the time-dependent signal intensity changes in the ROIs were converted into time-dependent concentration changes of Gd-DTPA as previously described [4]. Using the single compartment two-input kinetic model, distribution volume (DV) and mean transit time (MTT) of the contrast agent and portal fraction of hepatic blood inflow (PF) were calculated via a non-linear least-square fitting algorithm [4,5] written in MATLAB^{TM}.

Histopathology: Following MRI scans, the livers were harvested and stained with hematoxalyn, eosin and Masson's trichrome. Livers were scored on a 0-5 scale for fibrosis and steatosis by examining Masson's trichrome-stained slides and HE-stained slides, respectively, according to the presence and severity of fibrosis, vacuolar change (hydrophic/fatty) and bile duct proliferation. The rats were classified for statistical analysis and pathology findings by weeks of CCl4 treatment (control, 2-3, 6-8 and 11-16 weeks). Representative Oil Red O-stained frozen sections of liver samples from each group were reviewed to confirm the steatosis evaluation.

RESULTS

Histopathology: The degree of fibrosis in treated rats increased over time (Fig.1a). All control rats (with and without oil) had fibrosis scores of 0 and therefore were grouped together (denoted as 'control'). One rat in the 6-8 week group and 13 rats in the 11-16 week group had histologic changes indicating cirrhosis. The severity of steatosis was highest in rats on CCl4 treatment for 6-8 weeks (Fig.1b). Among the oil-treated control rats, 2 rats given oil for 14 weeks had steatosis and as a result the mean steatosis score of the oil-treated control group was higher than that of the control group without oil. However, the difference between the two control groups was not statistically significant (p = 0.089).

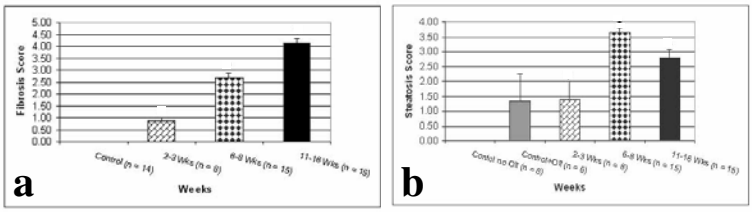

Fig.1 Fibrosis (a) and steatosis (b) scores in rats as a function of weeks of CCl4 treatment. The control rats with and without oil treatment are either collectively referred to as Control (a) or separately denoted as Control+Oil and 'Control no Oil', respectively (b). (mean ± standard error)

MRI: In accordance with the histologic findings the FWR was highest in rats on CCl4 treatment for 6-8 weeks (144% higher than control; p < 0.001) (Fig.2a). The control rats with oil had a higher FWR than the control rats without oil, but the difference was not statistically significant (p = 0.095). PF (Fig.2b) was moderately correlated with the duration of CCl4 treatment (r = - 0.483, p = 0.002). The DV of the 6-8 week group (Fig.2c) was only 54% of that of the control group (p < 0.001). The DV of the 11-16 week group was 67 % higher than that of the 6-8 week group (p = 0.004). DV was inversely correlated with FWR (r = - 0.581, p < 0.001) over 0-16 weeks of CCl4 treatment. There were no statistically significant differences in MTT between the control rats and those rats in the 2-3 and the 6-8 week groups (Fig.2d). However, the MTT of the 11-16 week group was 127 % higher than that of the 6-8 week group (p = 0.008).

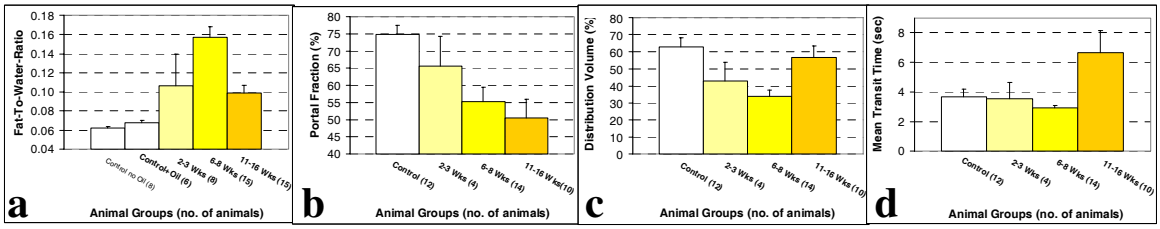

Fig.2 (a) Fat-to-Water Ratio (FWR), (b) Portal Fraction (PF), (c) Distribution Volume (DV), (d) Mean Transit Time (MTT) as a function of the duration of CCl4 treatment in rats. The control rats with and without oil treatment are either separately denoted as Control+Oil and 'Control no Oil', respectively (a) or collectively referred to as Control (b-d). (mean ± standard error)

DISCUSSION

The reversal of DV in synchrony with FWR between 6-8 weeks and 11-16 weeks of CCl4 treatment as well as their inverse correlation over 0-16 weeks of CCl4 treatment suggests that steatosis, in addition to fibrosis, may also influence MRI hepatic perfusion parameters by impinging sinusoids [2]. Thus, local distribution of the contrast agent is hindered in affected liver. The *sudden* increase in MTT in the 11-16 week group may also be due to the reduction in steatosis. That is, while DV is reduced, the slower diffusion of the contrast agent may not be depicted as an increase in MTT [5]. However, as fibrosis continues to progress, the severity of steatosis is reduced, and therefore the access of the contrast agent to the extravascular space may become less restricted leading to improved DV. Consequently, the slowed diffusion of the contrast agent in the extravascular space due to the increased collagen content therein can finally be manifested as prolonged MTT. The monotonic decrease in PF even when FWR is reversed may reflect the persistent portal hypertension in the end stage of liver diseases [2]. For non-invasive, reliable assessment of liver fibrosis using MRI and more relevant targeting of drug therapy, the factors influencing MRI-detectable hepatic perfusion parameters should be elucidated.

REFERENCES

[1] Benyon, *J Pediatr Gastroenterol Nutr* 27, 75, 1998. [2] Genecin, *The liver: biology and pathobiology*, 3rd ed., Raven Press, New York 1994, pp.1327-1341. [3] Hernandez-Munoz, *Hepatology* 26, 1100, 1997. [4] Marterne, *MRM* 47, 135, 2002. [5] Van Beers, *MRM* 49, 692, 2003. [6] Reeder, *MRM* 51, 35, 2004.

ACKNOWLEDGEMENTS

This work was supported by Pfizer Inc.