Evaluation of the Applicability of Magnetization Transfer Contrast as an Indicator of the Degree of Liver Fibrosis in Rats with Carbon Tetrachloride (CCl4)-Induced Liver Fibrosis/Cirrhosis

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

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

INTRODUCTION

Chronic liver disease, regardless of etiology, causes liver fibrosis, and its end-stage, cirrhosis, is one of the leading causes of morbidity and mortality in the world [1]. The pathogenesis of liver fibrosis is underpinned by the activation of hepatic stellate cells into a myofibroblast-like phenotype with a consequent overproduction of macromolecules (MMs) including collagen in the extracellular matrix (ECM) [2].

Magnetization transfer techniques (MT) [3] have been explored as a means of estimating the amount of ECM component or scar tissue in fibrotic/cirrhotic livers of rodent or humans [4,5,6]. However, due to limits in the ranges of disease severity and/or morphologic evaluations in previous studies, it is unclear, to the best of our knowledge, whether or not MT contrast (MTC) can specifically depict an increased amount of fibrous connective tissue in the affected liver. This study aims to assess the applicability of MTC as an indicator of the degree of liver fibrosis in rats with carbon tetrachloride (CCl4)-induced hepatic fibrosis/cirrhosis.

METHODS

Animal Preparation: The animal research protocol was approved by the Yale University Institutional Animal Care and Use Committee. A total of 52 male Wistar rats (Charles River Laboratories, Inc., Wilmington, USA) were used. CCl4 was injected intraperitoneally into treated animals mixed with vegetable oil (25 µl CCl4 in a 150 µl volume (1:6) at a frequency of three times per week for 2-16 weeks [7]. Six of the 14 rats in the control group received vegetable oil simutaneously at the same frequency. Rats underwent MRI scan 1-2 days after the last dose. Rats were anesthetized by subcutaneous injection of ketamine (80mg/kg; Fort Dodge Animal Health, Fort Dodge, USA) plus xylazine (10mg/kg; J. A. Webster, Inc. Sterling, USA) prior to MRI examination.

MRI: All images were collected on a 1.5 T Siemens MRI scanner with a phased-array 4-channel wrist coil (USA Instruments, Inc., Aurora, USA). A fast spin echo sequence was used with 6 pairs of saturation offset frequencies (f_{SAT}'s; ±800, ±1600, ±2400, ±3200, ±4000 and ±4800 Hz; bandwidth/duration of the saturation RF pulse = 800 Hz/7.65 ms). Other sequence parameters were: TR/TE = 2000/8.8 ms, FOV = 120 x 98 (matrix: 260 x 320), NEX = 3, flip angle = 90°/180°, 7-8 slices (4.5 mm thick). MT ratios (MTRs) were defined as 100 x $(S_o–S)/S_o$ where S and S_o are signal intensity with and without saturation, respectively.

Histopathology: After MRI, the livers were harvested. Two to four 4 mm thick pieces of the liver were sectioned at 5 microns and stained with hematoxalyn, eosin, and Masson's trichrome by routine methods. Livers were scored for fibrosis by examining Masson's trichrome-stained slides. Other parameters were obtained through analysis of HE-stained slides. Scores of 0 (within normal limits, absent), 1 (minimal, < 10%), 2 (mild, 10-20%), 3 (moderate, 23-30%), 4 (marked, 30-40%) and 5 (severe, > 40%) were assigned according to the severity of fibrosis. The rats were classified for statistical analysis and pathology findings by weeks of CCl4 treatment (control, 2-3, 6-8 and 11-16 weeks). Based on morphological findings rats were further divided into control, fibrosis (without cirrhosis) and cirrhotic groups.

RESULTS

Histopathology: The degree of fibrosis in treated animals increased over time (Fig.1). All control rats (with and without oil) had fibrosis scores of 0 and therefore were grouped together (denoted as control). Fourteen rats (1 from the 6-8 week group and 13 from the 11-16 week group) had histologic changes indicating cirrhosis. The 13 cirrhotic rats of the 15 rats in the 11-16 week group had fibrosis scores of ≥ 4.

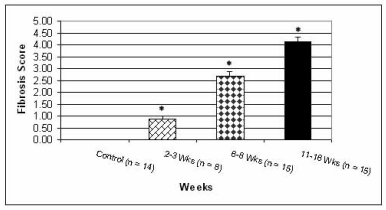

Fig.1 *Fibrosis scores in rats as a function of weeks of CCl4 treatment.(mean ± standard error)*

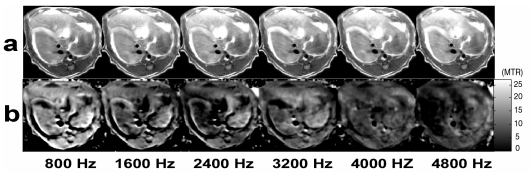

Fig.2 *Representative MT images of the liver of a normal rat with the saturation offset frequencies of 800-4800 Hz with an 800 Hz step size (a). The calculated MT ratio (MTR) images are shown in (b).*

MRI: Figure 2 displays representative MT images of the liver of a normal rat (a) and the calculated MTR images (b). The highest MTRs were obtained at ± 800 Hz and were in the range of 13-17. There were no significant differences in MTRs between the control rats with and without oil for all f_{SAT}'s (data not shown; $0.073 < p < 0.916$). Figure 3(a) illustrates the MTRs (normalized to that of the 11-16 week group for each of the figures of merit) as a function of weeks on CCl4-treatment at f_{SAT}'s of 4000 (MTR_{4000}) and 4800 Hz (MTR_{4800}) where the differentiation between the animal groups is best described. The sum of MTRs over all positive f_{SAT}'s ($MTR_{pos.sum}$) and over all f_{SAT}'s (both positive and negative offset; $MTR_{all.sum}$) are also shown. For all figures of merit, MTRs decrease until the duration of the CCl4-treatment reaches 6-8 weeks and then elevate slightly higher than the normal level by 11-16 weeks of CCl4-treatment. The same trend was observed in all individual (both positive and negative) f_{SAT}'s as represented by the changes in $MTR_{pos.sum}$ and $MTR_{all.sum}$. The MTR_{4800}, $MTR_{pos.sum}$ and $MTR_{all.sum}$ are inversely correlated with the duration of CCl4-treatment over 0-8 weeks ($-0.423 < r < -0.408$; $0.020 < p < 0.025$). For all figures of merit in Fig.3(b) (MTRs normalized to that of the cirrhotic group) there were significant differences between the fibrosis and the cirrhotic groups ($0.014 < p < 0.047$), and, although not statistically significant, the cirrhotic group was slightly higher (5-7%) than the control group. The distribution of MM content was to a large extent symmetric about the on-resonance frequency at all pairs of the mirrored f_{SAT}'s. As a consequence, the variation of $MTR_{pos.sum}$ as a function of disease severity was almost identical to that of $MTR_{all.sum}$ as shown in Fig.3.

DISCUSSION

The *inverse* correlation between MTRs and the duration of CCl4 treatment over 0-8 weeks suggests that the variations in MTR are not specific to ECM components. The underlying mechanism of the initial decrease of MTR followed by its reversal is unclear due to complicated pathologic changes and disease progression in affected livers, which may altogether compete against the increasing ECM. Due to the reversed MTR in cirrhotic liver, different conclusion could be drawn if no morphological distinction is made between fibrosis with and without cirrhosis. Although our results may be specific to the species and the animal model, in many respects the response in rats given CCl4 mirrors the pattern of disease seen in human fibrosis and cirrhosis [8]. The slightly higher MTR in cirrhotic liver with respect to control found in our study is in line with a previous human study with cirrhotic patients [5]. As the changes in MTR over the f_{SAT} range of ±800 ~ ±4800 Hz are symmetric and synchronous, a similar observation but higher MTC would be obtained with a larger bandwidth of the saturation RF pulse.

In conclusion, our results do not support the applicability of MTC as a specific indicator of the increased fibrosis in diseased liver. Nonetheless, MTC may still allow for the differentiation of liver fibrosis with and without cirrhosis. The decreasing MTR prior to the development of cirrhosis may also be a useful measure of the severity of fibrosis.

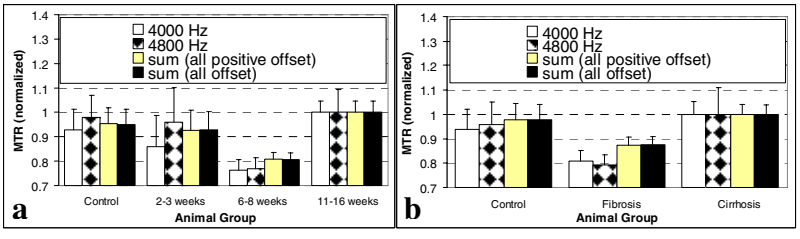

Fig.3 *(a): MT ratios (MTRs) of rats at saturation offset of 4000 and 4800 Hz, and the sum of MTRs over all positive offset and over all (positive and negative) offset as a function of weeks of CCl4 treatment. (b): results after resorting the rats into control, fibrosis (without cirrhosis) and cirrhotic groups. (for each of the figures of merit, MTR normalized to that of the 11-16 weeks (a) or of the cirrhotic group (b))*

REFERENCES

[1] Lotersztajn, *Annu Rev Pharmacol Toxicol* 45, 605, 2005. [2] Benyon, *J Pediatr Gastroenterol Nutr* 27, 75, 1998. [3] Guivel-Scharen, *JMR* 133, 36, 1998. [4] Aisen, *MRM* 31, 551, 1994. [5] Chen, *JMRI* 9, 257, 1999. [6] Guo, *JMRI* 18, 321, 2003. [7] Hernandez-Munoz, *Hepatology* 26, 1100, 1997. [8] Constandinou, Chapter 14, Vol. 117 in: *Fibrosis Research: Methods and Protocols*, edited by Varga et al, Humana Press Inc., Totowa, NJ 2005, pp237-250

ACKNOWLEDGEMENTS

This work was supported by Pfizer Inc.