Characterization of Liver Fibrosis by 1H- and 31P-MRS in CCl4-treated Rats

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INTRODUCTION

Liver fibrosis is a pathologic change caused by chronic liver damage, and its end-stage, cirrhosis, is one of the leading causes of morbidity and mortality in the world [1]. Nonetheless, liver biopsy remains the current gold-standard for staging the disease. To address this important clinical issue a variety of different MR imaging techniques have been explored as a non-invasive means of assessing liver fibrosis [2]. The evaluation of the potential diagnostic value of MR spectroscopy techniques has also been reported. However, the majority of those previous *in-vivo* MRS studies were conducted by using either ^{1}H- or ^{31}P-MRS. Thus, the performance and potential advantage of the combination of ^{1}H- and ^{31}P-MRS techniques in the in-vivo assessment of liver fibrosis is yet to be explored, which may potentially further extend the clinical applicability of MRS. In this report we have characterized liver fibrosis in CCl4-treated rats by using both ^{1}H- and ^{31}P-MRS, and examined the potential diagnostic efficacy of the technique.

MATERIALS AND METHODS

Animal Preparation: Fibrosis was induced in 27 male Sprague-Dawley 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 2-10 weeks [3]. Seven of the 14 control rats received pure vegetable oil at the same frequency.

MRS: All MRS spectra were collected on a 9.4 T Bruker Biospec scanner. For ^{1}H-MRS, a volume coil (Bruker, Germany) was used for both RF transmission and signal reception. Liver ^{1}H spectra were collected by using a STEAM sequence (TE/TM/TR = 2.2/20/5000 ms, spectral width = 5000 Hz, number of data points = 2048) from a total of three voxels (4x4x4 mm^3) in order to account for potential liver heterogeneity. For each voxel, spectra were collected without (number of averages = 32) and with (number of averages = 128) water-suppression for the quantification of choline-containing-compounds (CCC) relative to water and other metabolites relative to CCC, respectively. For ^{31}P-MRS, a double-tuned ^{1}H/^{31}P surface coil (Bruker, Germany) was used for both transmission and reception. Liver ^{31}P spectra were collected by using a pulse-acquire sequence with a nominal flip angle of 90^{o} (TR = 6000 ms, spectral width = 10 kHz, number of data points = 2048, number of averages = 128).

Data Analysis: All spectra were processed and analyzed by using Topspin software (Bruker, Germany; v 2.0). An exponential line broadening of 5 and 7 Hz was applied to ^{1}H- and ^{31}P spectra, respectively, followed by phase and baseline corrections. For both ^{1}H and ^{31}P spectra, peaks were assigned according to ref. [4], and the areas were estimated as a measure of metabolite content. For ^{1}H spectra, first, CCC/water was estimated from the spectra acquired without water-suppression, and then sugar and glycogen (Glyc) content were estimated with respect to CCC from the water-suppressed spectra. For ^{31}P spectra, those peaks of interest were quantified with respect to that of phosphocreatine (PCr). They were adenosine triphosphate (ATP), inorganic phosphate (Pi), phosphomonoester (PME; mainly phosphocholine (PC) + phosphoethanolamine (PE)), and phosphodiester (PDE; mainly glycerophosphocholine (GPC) + glycerophosphoethanolamine (GPE)).

Histopathology: Following MRS scans, the livers were harvested and stained with hematoxylin, eosin and Masson's trichrome. Livers were scored on a F0-F4 scale according to the presence and severity of fibrosis by examining Masson's trichrome-stained slides. The rats were classified for statistical analysis into control, control with oil, F1-2 and F3-4 groups.

RESULTS

Histopathology: The two control groups with and without oil did not differ in fibrosis score (p = 1.000) and therefore were grouped together (denoted as 'control' (n = 14)). There were 10 rats in the F1-2 group with the mean fibrosis score of 1.70±0.48. There were 17 rats in the F3-4 group with the mean fibrosis score of 3.12±0.08. Two rats in the F3-4 group had histologic changes indicating cirrhosis.

MRS: Representative ^{1}H (water-suppressed) and ^{31}P MRS spectra are shown in Fig.1. CCC/water level did not differ between the three rat groups. Sugar/Glyc and PME were mildly correlated with fibrosis scores (r = 0.323 (p = 0.034) and r = 0.327 (p = 0.037), respectively). As shown in Fig.2, both F1-2 and F3-4 groups had higher sugar levels than control (p = 0.033 and 0.007, respectively). Also, there was a trend of reduced Glyc in rats with increased disease severity. Consequently, sugar/Glyc better differentiates between control and the two treated groups (p = 0.021 (F1-2) and p = 0.001 (F3-4)). However, the separation between F1-2 and F3-4 was achieved only by PME/PDE level (p = 0.01). None of those ^{1}H-MRS measures allowed for that separation. In addition, PME level allowed for the differentiation between control and F3-4 (p = 0.02). The separation between control and F1-2, however, was not possible with these ^{31}P-MRS measures alone.

DISCUSSION

The invariant CCC/water level may be explained at least in part by the invariant content of GPC which is known to be one of the major contributors to the CCC resonance [5]. Unchanged water and CCC content in the progression of liver fibrosis was also reported previously [6]. Thus, CCC may also be used as an internal reference as in this study. The changes in the sugar and sugar/Glyc level observed herein are most likely due to the altered glucose metabolism in the diseased liver [7]. The gradual increase of the PME level manifests its role as a cell membrane precursor. Therefore, given that PDE is membrane breakdown products, the increased PME/PDE level in the progression of the disease from F1-2 to F3-4 indicates that the livers turned into an active regeneration mode in response to the insult [4]. The level of glutamate/glutamine complex (Glx) resonating at ~ 2.3 ppm in ^{1}H MRS spectra is also often investigated as a potential biomarker of liver diseases [6]. However, due to strong lipid signal that was observed for some of the rats (e.g., Fig. 1(a)) and resulting contamination of that spectral region, quantification of Glx was excluded. The increased Pi/ATP level observed in the previous in vitro study with cirrhotic rats [4], which was attributed to cellular hypoxia resulting from impaired liver function, was not seen in this study. Such discrepancy may be due to the fact that there were only two cirrhotic rats in this study.

In summary, the differentiation between the three animal groups in this study was achieved only if the combination of the ^{1}H- and ^{31}P-MRS measures was used. Therefore, our study demonstrates the advantage and feasibility of using both ^{1}H- and ^{31}P-MRS techniques in the assessment of liver fibrosis in vivo.

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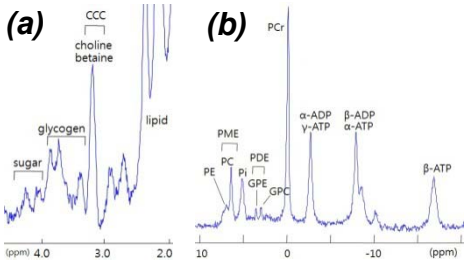
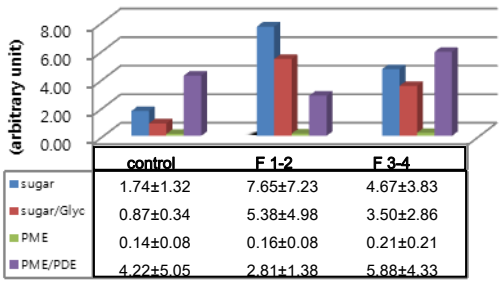


Fig.1 *Representative (a) water-suppressed ^{1}H MRS spectrum and (b) ^{31}P MRS Spectrum*



	control	F 1-2	F 3-4
sugar	1.74±1.32	7.65±7.23	4.67±3.83
sugar/Glyc	0.87±0.34	5.38±4.98	3.50±2.86
PME	0.14±0.08	0.16±0.08	0.21±0.21
PME/PDE	4.22±5.05	2.81±1.38	5.88±4.33

Fig.2 *Relative content of sugar, sugar/Glyc, PME and PME/PDE for the control, F1-2 and F3-4 groups*