Noninvasive Monitoring of CCl4 Induced Acute and Chronic Liver Damage in Rat by SQ and TQF 23Na MRI

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Introduction
Liver diseases are the eighth leading cause of death in the United States, and are expected to become an even more significant problem because of our aging population, obesity, diabetes, and alcohol consumption. The ability to noninvasively detect and monitor the severity and progression of liver diseases, especially chronic liver diseases, is a major priority in clinical area. The transmembrane sodium gradient plays many important roles in normal hepatocellular functions and could be disrupted in disease states due to compromised cellular integrity and energy status. The current study evaluated the ability of single quantum (SQ) and triple quantum filtered (TQF) 23Na MRI techniques to monitor the severity and progression of liver injury by acute and chronic CCl4 intoxication of rats. In addition, shift reagent (SR)-aided 23Na and 31P MRI techniques were employed to examine the mechanisms behind the changes in SQ and TQF 23Na MRI following CCl4 intoxication.

Methods
In acute liver damage model rats were given a single oral dose of 5 ml/kg body weight (BW) of CCl4 and corn oil mixture (ratio 1:1). SQ and MQF 23Na MRIs were acquired before and 24 hours after the treatment. In the chronic liver damage model, rats were given 1 ml/kg BW of CCl4, and corn oil mixture (ratio 1:1) orally twice per week for eight weeks. 23Na MRIs were acquired before treatment and every two weeks during the treatment. All MRI and MRS experiments were performed with a Varian 9.4 T 31 cm horizontal bore system. A 5 mm slotted tube resonator tuned to 106 MHz was used for SQ and TQF 23Na MRI. A 10 mm diameter tube containing 50 mM NaCl was placed inside the coil. The live SQ and TQF 23Na signal intensities (SIs) are reported relative to the reference. 23Na MRI was acquired with a 3D gradient-echo (GE) imaging sequence and the following parameters: 180 µs non-selective excitation radio-frequency (RF) pulse, 50 ms repetition time (TR), 4.6 ms echo time (TE), 64x64x16 data points over a 60x60x30 mm FOV, and 10 min data collection time. TQF 23Na MRI was acquired using a 3-pulse TQ filter followed by a GE imaging sequence. A preparation time of 4.5 ms and an evolution time of 10 µs was used. The other imaging parameters were the same as used for SQ 23Na MRI, except a TR of 100 ms and a data size of 64 x 32 x 8 were used. SR-aided 31Na and 31P MRS experiments were performed after the MRI experiments to measure the relative intra- and extracellular spaces (rECS and rECS, respectively) and intra- and extracellular Na+ concentrations ([Na+]i and [Na+]e, respectively). Rats were surgically prepared for TmDOTP infusion through the jugular vein and placement of the surface coil over the exposed liver. A surface coil (ø = 2 cm) tunable to 106 MHz for 23Na and 163 MHz 31P spectra was used. After the MRS experiments, rats were sacrificed and the liver tissue was fixed for histological examination.

Results
Representative trans-axial sections from 3D SQ and TQF 23Na MR images of the rat liver before and 24 hours after the acute CCl4 treatment are shown in Figure 1. The average SQ 23Na SI of the rat liver relative to the reference increased from 0.60±0.03 to 1.10±0.02 (p<0.05) and TQF 23Na SI increased from 0.35±0.11 to 0.96±0.12 (p<0.05) 24 hours after CCl4 treatment. The changes in SQ and TQF 23Na MRI SI for the control and chronic CCl4 treated rats over the 8-week period are shown in Figure 2. For the control group the average SQ 23Na SI decreased from 0.77±0.05 to 0.51±0.02 (p<0.05) and the average TQF 23Na SI decreased from 0.37±0.08 to 0.23±0.02 (p<0.05) over the 8 weeks. For the chronic CCl4 treatment group SQ 23Na SI remained relatively constant from week 0 (0.69±0.03) to week 6 (0.70±0.01) but afterwards increased to 0.87±0.03 (p<0.05) on week 8. At the same time, the average TQF 23Na SI progressively increased from 0.32±0.03 before CCl4 treatment to 0.40±0.02 at week 2, 0.42±0.02 at week 4, 0.46±0.02 at week 6 to 0.52±0.02 at week 8. Figure 3 shows a representative set of in vivo 23Na spectra from a control rat, an acute CCl4 treated rat and a chronic CCl4 treated rat. As compared to the control, the Na+ signal is higher in the acute CCl4 treated rat but the Na+ is higher in chronic CCl4 treated rat. The results of quantification of all the parameters measured by SR-aided 23Na and 31P experiments are shown in Table 1. In the acute CCl4 group, the total tissue sodium concentration ([Na+]i) and [Na+]e increased, ATP/Pi, decreased but rECS and pH did not change compared to the control group. In the chronic CCl4 group, rECS and [Na+]i increased, but [Na+]e, ATP/Pi, and pH did not change significantly compared to the control group. Histological slides of rat livers are shown in Figure 3. Acute CCl4 treatment induced inflammatory response in centrilobular regions, like fatty deposition, neutrophils infiltration and coagulative necrosis. On the other hand, chronic CCl4 treatment induced fibrosis shown with abundant collagen bundles.

Discussion
SQ 23Na MRI SI measures total tissue sodium and TQF MRI SI measures sodium ions transiently bound to macromolecules, which are mainly from intracellular space in the normal liver [1] but could also be from extracellular space in diseased states, like fibrosis. The results of SR-aided 23Na MRS and histology experiments show that the large increase in TQF 23Na MRI in the acute CCl4 group was due to an increase in [Na+]i caused by compromised cellular energy status and damaged cellular membrane integrity. In contrast the increase in TQF 23Na MRI in the chronic CCl4 group was due to overproduction of extra-cellular macromolecules and increased rECS.

Conclusion
SQ and TQF 23Na MRI are sensitive to cellular and tissue damage caused by acute and chronic CCl4 exposure. TQF 23Na MRI may be more useful in detecting the severity and progression of liver damage than SQ 23Na MRI because TQF signal depends on [Na+]i and accumulation of extracellular macromolecules, which are major patho-physiological changes in many liver diseases [2].

References