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

Y. Gao[1], J. James[1], and N. Bansal[1]

[1]Radiology, Indiana University School of Medicine, Indianapolis, Indiana, United States

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 hepatocelluar function, 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) ^{23}Na MRI techniques to monitor the severity and progression of liver injury by acute and chronic CCl_4 intoxication of rats. In addition, shift reagent (SR)-aided ^{23}Na and ^{31}P MRS techniques were employed to examine the mechanisms behind the changes in SQ and TQF ^{23}Na MRI following CCl_4 intoxication.

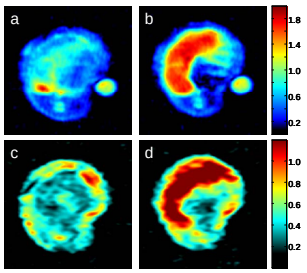

Figure 1. Representative slices from a 3D SQ (a, b) and TQF (c, d) ^{23}Na MRI of rat liver before CCl_4treatment (a, c) and 24 hours after CCl4 treatment (b, d).

Methods

In acute liver damage model rats were given a single oral dose of 5 ml/kg body weight (BW) of CCl_4 and corn oil mixture (ratio 1:1). SQ and MQF ^{23}Na MRIs were acquired before and 24 hours after the treatment. In the chronic liver damage model, rats were given 1 ml/kg BW of CCl_4 and corn oil mixture (ratio 1:1) orally twice per week for eight weeks. ^{23}Na MRIs were acquired before treatment and every two weeks during the treatment. All MRI and MRS experiments were performed with a Varian 9.4T 31 cm horizontal bore system. A 50 mm slotted tube resonator tuned to 106 MHz was used for SQ and TQF ^{23}Na MRI. A 10 mm diameter tube containing 50 mM NaCl was placed inside the coil. The liver SQ and TQF ^{23}Na signal intensities (SIs) are reported relative to the reference. SQ ^{23}Na 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), 64×64×16 data points over a 60×60×30 mm FOV, and 10 min data collection time. TQF ^{23}Na 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 were used. The other imaging parameters were the same as used for SQ ^{23}Na MRI, except a TR of 100 ms and a data size of 64 x 32 x 8 were used. SR-aided ^{23}Na and ^{31}P MRS experiments were performed after the MRI experiments to measure the relative intra- and extracellular spaces (rICS and rECS, respectively) and intra- and extracellular Na^+ concentrations ($[Na_i^+]$ and $[Na_e^+]$, respectively). Rats were surgically prepared for $TmDOTP^{5-}$ infusion through the jugular vein and placement of the surface coil over the exposed liver. A surface coil (φ = 2 cm) tunable to 106 MHz for ^{23}Na and 163 MHz ^{31}P 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 ^{23}Na MR images of the rat liver before and 24 hours after the acute CCl_4 treatment are shown in Figure 1. The average SQ ^{23}Na 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 ^{23}Na SI increased from 0.35±0.11 to 0.96±0.12 (p<0.05) 24 hours after CCl_4 treatment. The changes in SQ and TQF ^{23}Na MRI SI for the control and chronic CCl_4 treated rats over the 8-week period are shown in Figure 2. For the control group the average SQ ^{23}Na SI decreased from 0.77±0.05 to 0.51±0.02 (p<0.05) and the average TQF ^{23}Na SI decreased from 0.37±0.08 to 0.23±0.02 (p<0.05) over the 8 weeks. For the chronic CCl_4 treatment group SQ ^{23}Na 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 ^{23}Na SI progressively increased from 0.32±0.03 before CCl_4 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* ^{23}Na spectra from a control rat, an acute CCl_4 treated rat and a chronic CCl_4 treated rat. As compared to the control, the Na^+_i signal is higher in the acute CCl_4 treated rat but the Na^+_e is higher in chronic CCl_4 treated rat. The results of quantification of all the parameters measured by SR-aided ^{23}Na and ^{31}P experiments are shown in Table 1. In the acute CCl_4 group, the total tissue sodium concentration ($[Na^+_t]$) and $[Na^+_i]$ increased, ATP/P_i decreased but rECS and pH did not change compared to the control group. In the chronic CCl_4 group, rECS and $[Na^+_t]$ increased, but $[Na^+_i]$, ATP/P_i and pH did not change significantly compared to the control group. Histological slides of rat livers are shown in Figure 3. Acute CCl_4 treatment induced inflammatory response in centrilobular regions, like fatty deposition, neutrophils infiltration and coagulative necrosis. On the other hand, chronic CCl_4 treatment induced fibrosis shown with abundant collagen bundles.

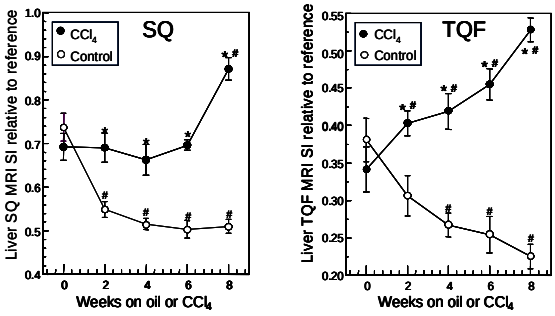

Figure 2. SQ and TQF ^{23}Na SIs of rat livers over 8-week CCl_4 treatment (p<0.05, * - vs. control, # - vs. previous time point)

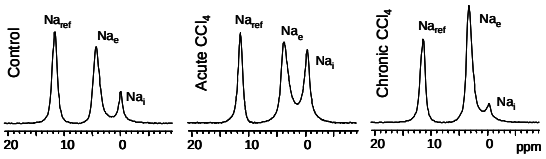

Figure 3. ^{23}Na Spectra from reference bulb and liver of control, acute CCl_4 treated rat, and chronic CCl_4 treated rat

Table 1. Parameters measured by *in vivo* SR-aided ^{23}Na and ^{31}P MRS in acute and chronic CCl_4 intoxication experiment (* - p<0.05, vs. Control)

	Acute CCl_4 experiment		Chronic CCl_4 experiment	
	Control	CCl_4	Control	CCl_4
rECS	0.24 ± 0.02	0.26 ± 0.01	**0.19 ± 0.03**	**0.28 ± 0.03 ***
$[Na^+_t]$, mM	**32 ± 2**	**46 ± 4 ***	**35 ± 4**	**52 ± 7 ***
$[Na^+_i]$, mM	17 ± 2	49 ± 7 *	18 ± 2	18 ± 2
ATP/Pi	**1.24 ± 0.08**	**0.94 ± 0.10 ***	1.24 ± 0.12	1.35 ± 0.07
pH	7.33 ± 0.02	7.29 ± 0.01	7.36 ± 0.04	7.29 ± 0.03

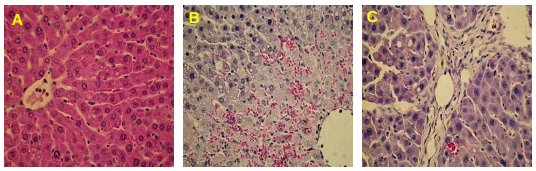

Figure. 4. Histological examination of rat liver slices by H&E staining in 400× magnification (A) normal control (B) acute CCl_4 induced liver injury, (C) chronic CCl_4 induced liver injury

Discussion

SQ ^{23}Na MRI SI measures total tissue sodium and TQF MRI measures sodium ions transiently bound to macromolecule, 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 ^{23}Na MRS and histology experiments show that the large increase in TQF ^{23}Na MRI in the acute CCl_4 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 ^{23}Na MRI in the chronic CCl_4 group was due to overproduction of extra-cellular macromolecules and increased rECS.

Conclusion

SQ and TQF ^{23}Na MRI are sensitive to cellular and tissue damage caused by acute and chronic CCl_4 exposure. TQF ^{23}Na MRI may be more useful in detecting the severity and progression of liver damage than SQ ^{23}Na 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: [1] Seshan *et al. MRM* 38:821 -27 (1997) [2] Weber *et al. Crit Rev Toxicol*. 33:105-36 (2003)