In Vivo Proton MRS of Liver in an Experimental Liver Fibrosis Model

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Introduction

Carbon tetrachloride (CCl_4) intoxication is a well-characterized, reproducible and the most commonly used experimental animal model of liver fibrosis. It has been widely studied with respect to hstological, biochemical, cellular, and molecular changes associated with development of fibrosis[1,2]. By interfering hepatic energy metabolism and protein synthesis, CCl_4-induced hepatotoxicity can lead to triglyceride accumulation, mitochondrial injury, and necrosis[3]. While proton magnetic resonance spectroscopy (^{1}H MRS) can provide insights into in vivo liver metabolism noninvasively, detailed in vivo MRS study of CCl_4-induced liver fibrosis model has been limited. The aim of this study was to characterize early metabolic changes in CCl_4-induced liver fibrosis in rodents by means of single-voxel ^{1}H MRS.

Methods

Animal Procedures: Liver fibrosis was induced in male Sprague-Dawley (SD) rats (220-260 g; $N = 8$) by subcutaneous injection of 1:1 mixture of CCl_4 in olive oil at a dose of 0.2mL/100g of body weight twice a week for 4 weeks[2,4]. Liver fibrosis in animals was confirmed by hematoxylin-eosin (H&E) staining. Normal male SD rats (220-260 g; $N = 8$) were used as controls. ^{1}H MRS was performed in the CCl_4-injected animals at 2 and 4 weeks after the start of CCl_4 injection.

In Vivo Liver MRS: ^{1}H MRS experiments were performed on a 7T Bruker MRI scanner with a 60 mm RF Tx/Rx quadrature resonator. Each animal was anesthetized with ~1.5 % isoflurane and maintained at about 36.5°C with respiratory monitoring. Scout images were first acquired in three orthogonal planes with a FLASH sequence. A 5×5×5 mm^3 voxel was chosen within a homogeneous liver parenchyma to avoid large blood vessels. 1st- and 2nd-order localized automatic shimming was first performed within the voxel until a FWHM <50 Hz was achieved in the water peak. The water signal was then suppressed by variable power RF pulses with optimized relaxation delays (VAPOR). Outer volume suppression (OVS) combined with respiratory-triggered point-resolved spectroscopy (PRESS) sequence was used for MRS acquisition, with TR = 2 respiratory cycles (~2.0-2.5 s), TE = 15 ms, BW = 4 kHz, 2048 data points, 256 averages and total scan time of ~10 min.

Data Analysis: The raw data were zero-filled to 8192 data points, apodized with a 2-Hz exponential filter, Fourier transformed, 0th- and 1st-order phase corrected and baseline corrected. Signal integrals of lipid (integral sum of $–CH_3$, $(–CH_2–)_n$, and –CH=CH– at 0.9, 1.3 and 5.3 ppm, respectively), glumatine and glutamate complex (Glx; at 2.2 ppm), choline-containing compounds (CCC; at 3.2 ppm)[5] were manually quantified by areas under peaks. The lipid-to-CCC and Glx-to-CCC ratios were measured by dividing peak area of metabolite by peak area of CCC. The relative saturated and unsaturated fatty acid fractions were estimated by dividing peak areas of $(–CH_2–)_n$ and –CH=CH– by peak area of lipid, respectively. One-way ANOVA was employed to compare differences in ratios of peak areas in different groups, with P values less than 0.05 considered statistically significant.

Results

Fig. 1 shows the typical liver ^{1}H MRS spectra from a normal control animal and an animal scanned at 2 and 4 weeks after CCl_4 injection with a typical voxel placement shown in the anatomical image. It was consistently observed that all animals with CCl_4 injection exhibited substantial increase in all metabolites, except CCC, as compared with the normal control animals. Fig. 2 shows the lipid-to-CCC, Glx-to-CCC, $(–CH_2–)_n$-to-lipid, and –CH=CH–-to-lipid ratios in normal ($N = 8$) and CCl_4-injected animals ($N = 8$). Significant differences ($P < 0.01$) were found for lipid-to-CCC, Glx-to-CCC and $(–CH_2–)_n$-to-lipid ratios.

Discussions and Conclusion

(**1**) As CCC is believed to represent the important constituents in phospholipid metabolism of cell membranes, similar CCC levels observed in CCl_4-injected and normal animals were expected because of the similar rate of cell turnover of hepatocytes in liver prior to the development of cirrhosis[6]. (**2**) As a result of the destruction of microsomal proteins by lipid peroxidation during toxic insult of CCl_4, hepatocytes are incapable of synthesizing lipoproteins that are needed to remove triglycerides in the cytoplasm[7,8]. Therefore, the lipid signal increase in animals with CCl_4 injection was probably due to fatty infiltration/fatty changes in hepatocytes, leading to increased triglyceride content[9,10]. (**3**) The increase in saturated fatty acid fraction may reflect the lipid-induced cell toxicity, which is worsened by saturated fatty acids[11,12]. (**4**) Hepatic glutamine metabolism in connection with urea synthesis plays a central role in nitrogen metabolism, in which glutamine represents a storage and transport form of glutamate and ammonia[13]. As in hepatic encephalopathy[14,15], the Glx signal increase in CCl_4-injected animals may result from increased glutamine synthesis due to compromised ammonia detoxification and subsequent hyperammonemia during liver dysfunction. (**5**) The discrepancies between the current MRS findings and those in human chronic hepatitis[16,17] likely reflect varying pathogenesis/etiology of liver fibrosis. In conclusion, our experimental results demonstrated that ^{1}H MRS at high field was useful in characterizing metabolic changes in liver, in particular those related to lipid and glutamine metabolism, after CCl_4 insult. ^{1}H MRS may be valuable in detecting fatty changes at early phase and other metabolic changes in human liver fibrosis associated with steatohepatitis.

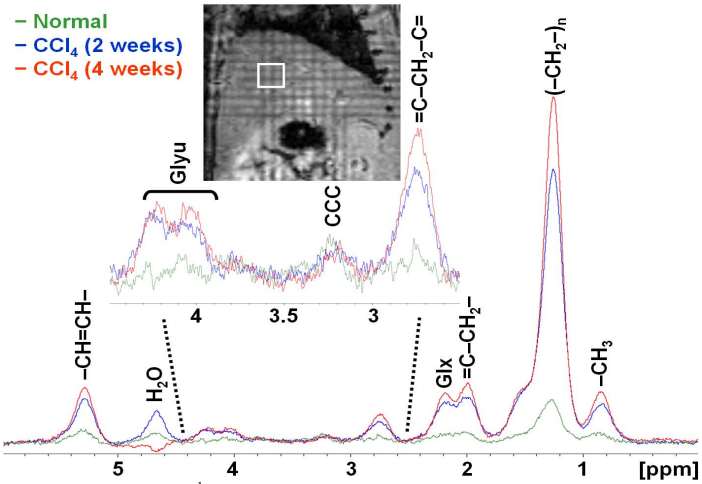

Fig. 1. Typical liver ^{1}H MRS spectra from a normal control animal and an animal scanned at 2 and 4 wks after CCl_4 injection. Animals with CCl_4 injection consistently showed markedly increase in various metabolites except CCC.

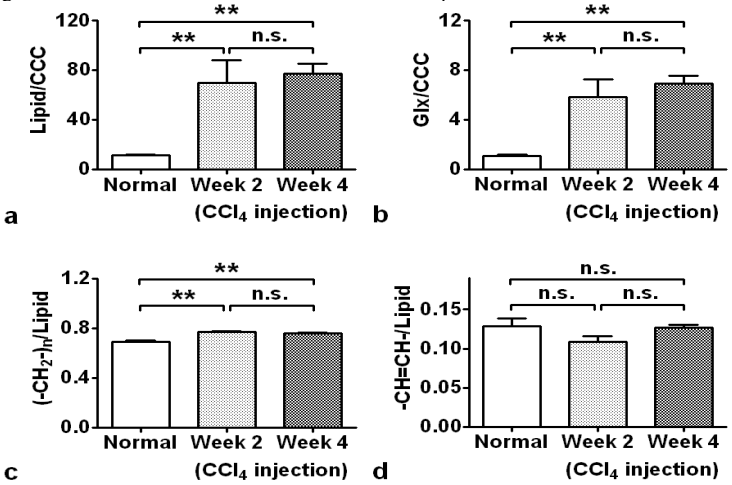

Fig. 2. (**a**) Lipid-to-CCC, (**b**) Glx-to-CCC, (**c**) $–CH_2–$-to-lipid, and (**d**) –CH=CH–-to-lipid ratios in normal animals and animals with CCl_4 injection of 2 and 4 wks. One-way ANOVA was performed with ** for $P < 0.01$ and n.s. for insignificance.

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