Investigation of early biochemical changes in liver fibrosis using an experimental mouse model

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TARGET AUDIENCE: Researchers interested in chronic liver diseases

PURPOSE: Hepatic fibrosis is associated with chronic liver injuries (metabolic, inflammatory, parasitic and toxins) resulting in cirrhosis and eventually leading to increased to risk of hepatocellular carcinoma[1-3]. It is characterized by the excessive accumulation of extracellular matrix (ECM) proteins. Accumulation of ECM, distorts the hepatic architecture by forming fibrous scar and its subsequent development resulting in regenerating hepatic nodules which defines cirrhosis. Image confirmation of chronic liver injury can be revealed at later stages of cirrhosis and neoplasm (HCC). During early stages of fibrosis cellular and molecular changes take place involving hepatic stellate cells, myofibroblast and ECM. Alterations in lipid composition play an important pathogenic role during the progression of liver fibrosis and cirrhosis. In this study we have investigated the longitudinal alterations in lipid composition and sodium content in hepatic fibrosis.

ANIMAL MODELS AND METHODS: All in vivo experiments were in compliance and approved by institutional animal care and use committee. Liver fibrosis was induced in male adult wild type (WT) mice (n=12) by intraperitoneal injection of 1:4 volume mixture of CCl4 in olive oil at a dose of 1ul of CCl4/gm of body weight, twice a week for 16 weeks. Control animals received equal volumes of olive oil. In vivo MR experiments with motion compensated respiratory gating were performed on a 7 T ClinScan MRI/MRS scanner (Bruker) equipped with a 72-mm volume resonator in combination with a 20-mm surface receive-only coil. Volume-localized PRESS sequence was employed on liver with TR=4 s, TE= 13 ms, NA 128, voxel volume=16 mm^3, SW= 3500 Hz, data points= 2048. Fat content from in vivo spectra were estimated using LC Model software[4]. Multidirectional DTI measurements were performed with b = 0, 700 s mm^{-2}, gradient directions-20, NA-4. ^{23}Na high resolution magic angle spectra (HRMAS) of liver tissue samples were acquired using a 9.4T Bruker Avance III spectrometer and a 4 mm HRMAS HX probe. Quantitative one-dimensional spectra were acquired with 16K data points, spin rate- 4K, sample temperature- 277 K, spectral width- 6355 Hz, relaxation delay-10 sec, NA-2000. Concentrations of sodium were estimated with reference to known concentration of sodium chloride. All ex vivo data were analyzed using TOPSPIN 3.0 software. Fatty liver samples were histologically analyzed by sirius red, hematoxylin and eosin staining.

RESULTS: **Figure 1** shows the in vivo spectra of liver acquired longitudinally at 0 week (baseline), 2, 7 and 16 weeks of CCl4 treatment. **Figure 2** shows the fat fraction of CCl4 treated and control groups of mice at different time points. The hepatic lipid content at baseline are similar for both CCl4 treated and control groups (0.131 and 0.129). After 2 weeks of CCl4 treatment the lipid content in CCl4 treated group (0.263 ±0.01) was significantly ($P < 0.001$) higher than control group (0.15 ±0.01). Lipid content in CCl4 treated mice progressively increased at 7 weeks (0.32 ± 0.02, $P < 0.05$) and 16 weeks (0.43 ± 0.02, $P < 0.001$) compared to control mice. **Figure 3A,B** shows the total saturated lipids (TSL) and mono-unsaturated lipids (MUL) content in CCl4 treated and control mice. **Figure 4** shows the ADC values measured from control and fibrotic livers at different time points of CCl4 treatment. The average ADC values of the fibrotic livers (0.74 ± 0.05 x 10^{-3} to 0.86 ± 0.08 x 10^{-3} mm^2/s) were significantly ($P < 0.001$) lower than control livers (1.17 ± 0.05 10^{-3} mm^2/s). **Figure 5** shows the ^{23}Na HRMAS spectra from cirrhotic and control livers. Sodium content in fibrotic livers was significantly ($P < 0.05$) higher (2.6 folds) compared to control livers. **Figure 6** shows the sirius red stained sections of cirrhotic and control livers at 7 and 16 weeks of CCl4 treatment.

Figure 1

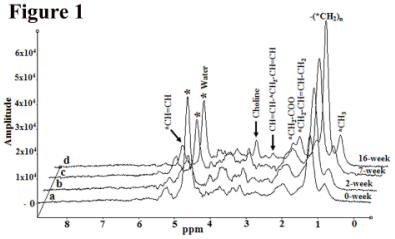


Figure 2

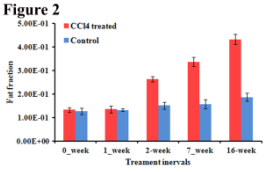


Figure 3A

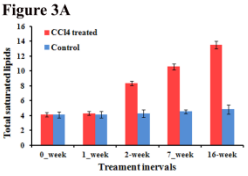


Figure 3B

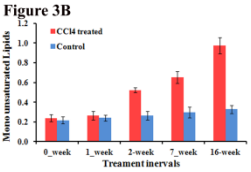


Figure 4

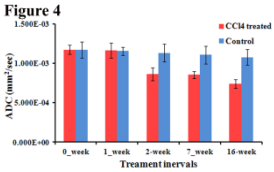


Figure 5

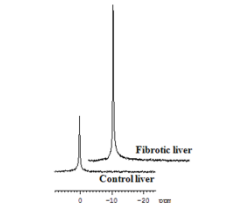
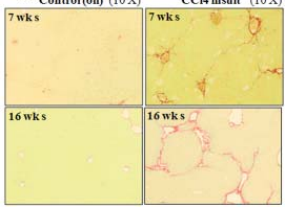


Figure 6



DISCUSSION: The longitudinal analysis showed significant increase in lipids for the CCl4 treated group at 2, 7 and 16 weeks indicating the establishment of fibrosis in the liver. Under fibrotic condition the hepatocytes are incapable of synthesizing lipoproteins in the cytoplasm due to destruction of microsomal proteins by lipid peroxidation[5-6] leading to increased accumulation of triglycerides in fibrotic tissue[7]. Both TSL and MUL did not differ significantly at 1 week after CCL4 treatment.. At 2, 7 and 16 weeks the TSL and MUL significantly increased ($P < 0.001$) in fibrotic livers compared to control livers. Increased incorporation of saturated lipids in the cell membranes increase the membrane rigidity resulting in lipid induced toxicity. The increased availability of hepatic saturated fatty acids in fibrotic/cirrhotic liver reduces hepatic fatty acid oxidation and triglyceride export. Increase in mono-unsaturated lipids is due to up regulation of stearoyl-CoA desaturase 1 (SCD1) which plays a crucial role in the pathogenesis of chronic liver disease in cirrhotic tissue. DTI measurements revealed the microstructural and pathological alterations in fibrotic liver tissues. Compromised cellular energy and damaged cellular membrane integrity lead to increase in extracellular sodium in fibrotic tissues. Histology showed the presence of multiple foci, lobulization and nodular fibrosis in CCl4 treated group. The bridging fibrosis between portal areas, fat vacuoles and collagen deposition were also seen in CCl4 treated livers.

CONCLUSIONS: Altered lipid metabolism was observed in mice within 2 weeks of carbon tetrachloride treatment. Lipid fraction, total saturated and mono-unsaturated lipids were higher in fibrotic livers indicating reduced fatty acid oxidation and triglyceride export compared to control livers. Sodium content in fibrotic livers was significantly higher than control livers with compromised cellular membrane integrity. Histological observations confirmed the presence of nodular and bridging fibrosis between portal areas. Lobulization characteristics were dominant in the fibrotic livers. Non invasive detection of early changes in fibrosis will be helpful in a clinical setting for design of therapeutic interventions.

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