QUANTITATIVE MRI OF LIVER FIBROSIS IN AN EXPERIMENTAL MOUSE MODEL

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INTRODUCTION

Liver fibrosis is a common response to chronic liver injury[1]. Early diagnosis of liver fibrosis could facilitate early interventions and treatments, thus prevent its progression to cirrhosis[2]. Conventional MRI has been employed to assess liver fibrosis; however the anatomical analysis has been found to be subjected to interobserver variability and limited in sensitivity and specificity[3]. MR elastography has shown promise in assessing liver fibrosis by measuring tissue stiffness[4]. Apparent diffusion coefficient measured by diffusion imaging has been used to characterize liver fibrosis[5,6]. However, the clinical utility of these advanced MRI techniques for staging liver fibrosis has yet to be established. Quantitative MRI has been used to characterize liver fibrosis[7-10]; however, correlation between relaxation times and fibrosis stage is still controversial. Recently, a preliminary human study has reported that liver T_2 value increases monotonically with increasing fibrosis stage[11]. Quantitative mapping of relaxation times can be routinely and reliably performed in standard scanners with rapid imaging capability and breath-holding/triggering techniques and hence may be valuable and robust in clinical settings. In this study, we aim to characterize the change in relaxation times longitudinally in a well-controlled experimental mouse model of liver fibrosis.

METHODS

Animal Preparation: Male adult C57BL/6N mice (22-25g; N=12) were prepared. Liver fibrosis was induced by subcutaneous injection of 1:3 mixture of carbon tetrachloride (CCl_4) in olive oil at a dose of 4μL/g of body weight twice a week for 4 weeks[12]. Quantitative MRI was performed in animals 1 day before, 2 and 4 weeks after CCl_4 administration. ***MRI:*** All MRI experiments were performed on a 7T Bruker MRI scanner using a 38-mm quadrature RF coil. Under inhaled isoflurane anaesthesia, the animal was kept warm under circulating water at 37°C. Each animal was placed in prone position with the abdomen fixed with adhesive tape to reduce respiratory. T_1 values were measured with a series of SE images with varying TRs=125,250,500,1000,2000,4000ms, TE=8ms, acquisition matrix =128×128, spatial resolution=0.23×0.23×2mm^3, NEX=1. Similarly, T_2 values were measured with multi-echo SE imaging sequences using TR=2000ms, TEs =8,16,24,32,40,48,56, 64ms, acquisition matrix=128×128, spatial resolution=0.23×0.23×2mm^3, NEX=1. ***Data Analysis:*** T_1 values were calculated by mono-exponential recovery fitting of the multi-TR SE signals on a pixel-by-pixel basis. Similarly, T_2 values were computed by mono-exponential fitting of the multi-echo SE signals on a pixel-by-pixel basis. A ROI was defined to encompass a large homogeneous liver region for T_1 and T_2 measurements. One-way ANOVA with Tukey's multiple comparison test was employed to compare the measurements between different time points of liver fibrosis. ***Histology:*** Animals were immediately sacrificed after MR examinations. Liver specimens were fixed in formalin, embedded in paraffin, sectioned and examined by light microscopy after standard H&E and Masson's trichrome staining.

RESULTS

Fig. 1 shows the typical T_1-weighted and T_2-weighted images, T_1 and T_2 maps of liver from one animal 1 day before, 2 and 4 weeks after CCl_4 insult. Fig. 2 shows the liver T_1 and T_2 values at different time points for all the animals studied. Fig. 3 shows the typical H&E and Masson's trichrome staining of normal liver and livers at 2 and 4 weeks after CCl_4 insult. Compared with normal liver, collagen deposition and intracellular fat vacuoles were consistently observed in livers with CCl_4 insult. Cell necrosis/apoptosis was evident in liver with 2-week CCl_4 insult, while collagen deposition was more pronounced in liver with 4-week CCl_4 insult.

DISCUSSIONS AND CONCLUSIONS

Intermittent administration of CCl_4 provides an established and widely used model of liver fibrosis in rodents by evoking a marked infiltration of inflammatory cells, thus mimicking the changes in chronic viral hepatitis-associated fibrosis[12,13]. The twice-weekly dosing can induce early stages of liver fibrosis and established fibrosis after 2 and 4 weeks of CCl_4 administration, respectively, in rodents[12]. This well-controlled CCl_4-induced liver fibrosis model allows the study of a homogeneous population of liver fibrosis. In this study, increased T_1 values observed in the current study were probably due to the increased hepatic water content of liver edema[9], while increased T_2 values likely resulted from the associated inflammatory changes of CCl_4 insult[12]. Moreover, the trend of T_2 increases observed in the current study was consistent with the recent preliminary human study[11]. Clinically, many diseases are characterized by inflammation and edema which may progress to fibrotic scarring, or cirrhosis[10]. Our results suggest that both relaxation times may serve as sensitive markers for liver fibrosis. Quantitative MRI may be valuable and robust in detecting liver fibrosis at early phase and monitoring its progression.

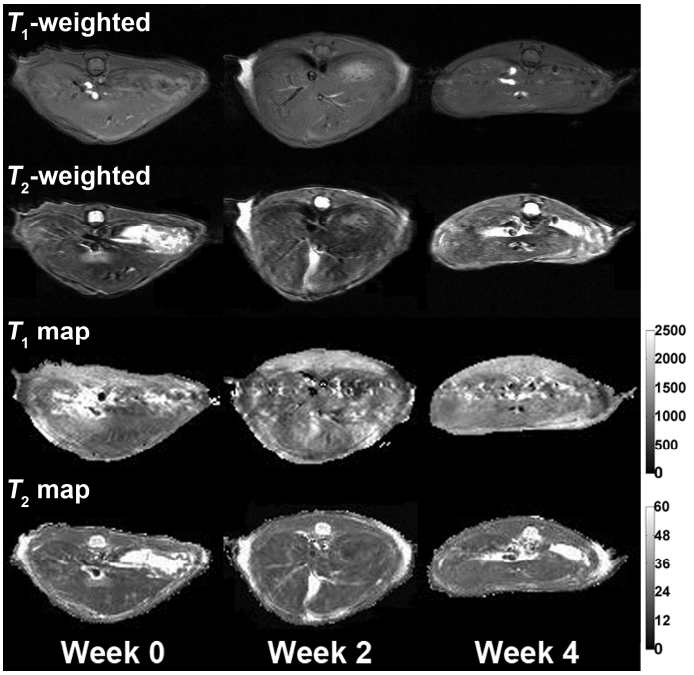

FIG. 1. T_1-weighted and T_2-weighted images, T_1 and T_2 maps from one animal at 0, 2 and 4 weeks after CCl_4 insult.

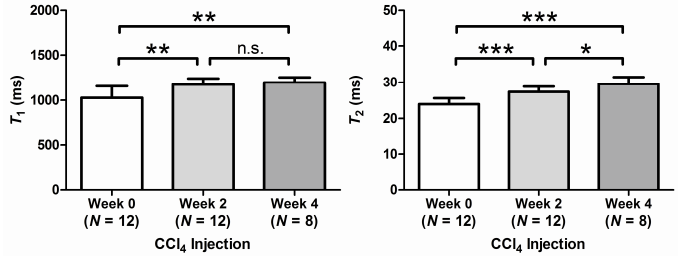

FIG. 2. Liver T_1 and T_2 values of animals at 0 (before insult), 2 and 4 weeks after CCl_4 insult. Error bars represent SD. One-way ANOVA was performed with * for p<0.05, ** for p<0.01, *** for p<0.001 and n.s. for insignificance.

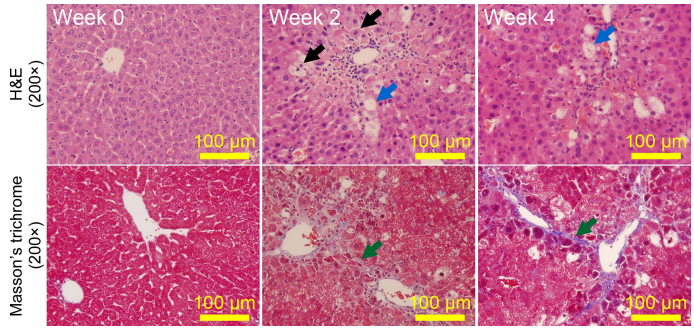

FIG. 3. Typical H&E staining (200×; top row) and Masson's trichrome staining (200×; bottom row) (**a**) normal liver, and livers subjected to (**b**) 2-week and (**c**) 4-week CCl_4 twice-weekly administration. Collagen deposition (green arrows), fat vacuoles (blue arrows), and cell necrosis/apoptosis (black arrows) were observed in the insulted livers.

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