Fibrous liver stiffness analysis using high frequency Magnetic Resonance Elastography at 7T on an ex vivo rat model. Feasibility and preliminary results

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Introduction

Multiple studies have reported on developments of hepatic MR Elastography (MRE) to quantitatively assess hepatic fibrosis by measuring the viscoelastic properties of the liver in animal models and humans [1-4]. No recent study has shown a close correlation between *ex vivo* liver samples and histological semi qualitative quantification of liver fibrosis. The purpose of this study is to evaluate the feasibility of a high-resolution MRE-assessed fibrous liver stiffness analysis in an *ex vivo* rat model with histological correlation.

Materiel and Method

Fibrosis was induced in Wistar male rats (~250g) using CCl4 intoxication. Each rat was injected with .1ml/100g of CCl4 diluted at 50% in olive oil twice a week. Each week 6 rats were anesthetized with a 4% isoflurane-oxygen gas mixture (400 mL/min initial dose) and sacrified by intraperitoneal injection of 1ml of sodium thiopental. Each liver was resected and a liver sample was obtained with a 19mm diameter punch. 48 rats were included to allow the analysis from 1 to 8 weeks after the beginning of liver fibrosis induction. A group of normal rats (n=8) was also sacrificed and liver samples were excised using the same procedure. Histological analysis of liver samples was performed with routine hematoxilin-eosin and picrosirius staining. Fibrosis was assessed using the METAVIR semi-quantitative score. MRE was performed on a horizontal 7 T imaging scanner (Pharmascan, Bruker, Erlangen, Germany). T2-weighted anatomical scans were performed to allow for proper slice positioning of the MRE scan. The vibration was generated by a toothpick placed in the center of the liver sample to induce a circular propagation. An electromagnetic shaker (Brüel & Kjaer, Nærum, Denmark) located outside the MR scanner was used to transmit mechanical vibrations via a flexible carbon fiber rod to the toothpick. Samples placed around the toothpick were always at the same height via a home-made support(Figure1). For each sample a steady-state MRE sequence was applied with a mechanical excitation frequency of 600 Hz and the following sequence parameter: 8 dynamics, 7 contiguous transverse slices with slice thickness of 0.4 mm, field of view = 25 mm × 25 mm, matrix size = 256 × 256, TE/TR = 12.5/270 ms and acquisition time of 138 seconds. The MRE sequence was acquired for the three spatial direction of motion in order to obtain volumetric images of the 3D propagating mechanical wave inside the sample. Data were reconstructed with an isotropic reconstruction technique and elasticity shear modulus (Gd) was extracted [5]. Regions of interest were drawn on the Gd parametric maps to obtain mean Gd on 3 consecutive slices for each sample (figure 2).

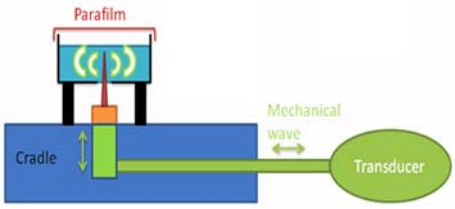

Figure 1 : Schematic of the bench used for sample characterization by MRE at 7T.

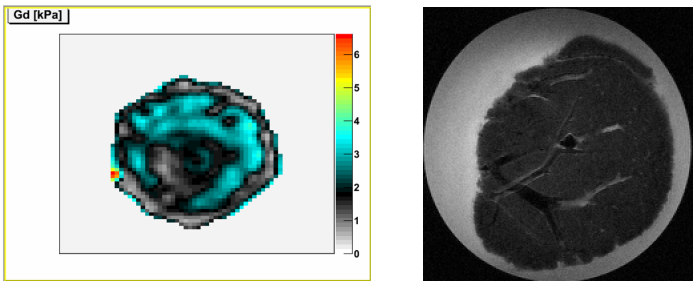

Figure2. A: Example of elasticity shear modulus (Gd) (left side). Anatomic view of a liver sample on T2-weighted imaging (right side)

Preliminary result and discussion

The mean shear modulus of normal rat livers was significantly lower than the mean Gd of fibrous livers at W3 (p=.01) From week 1 (W1) to week 3 (W3) we observed a 32% increase of Gd as presented in Figure 3A. This was closely correlated with the histological findings: F0 in normal rats as expected, F1 in W1 livers, F2 in W2 and fibrosis increase from F2 to early F3 in W3 (figure 3B).

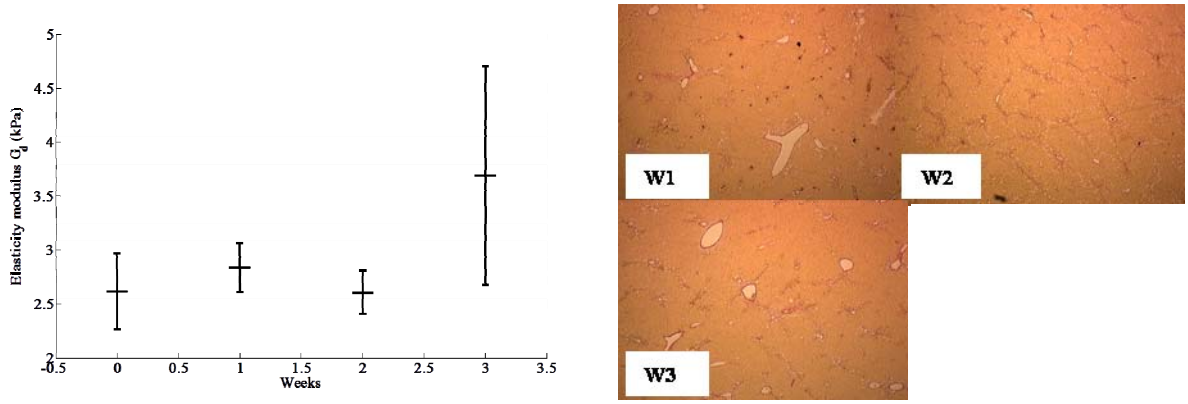

Figure 3: Normalized shear modulus (Gd) evolution during the 3 first weeks of fibrosis induction compared to normal livers (left side). B: Picrosirius staining evolution during the 3 first weeks of fibrosis induction (right side)

Conclusion and perspectives

In this study, we demonstrated that MRE can assess early stiffness modifications in a fibrosis induction rat model. It is expected that Gd will significantly increase in the next 5 weeks as fibrosis will be more intense. In a next step, adjacent slices of a macroscopic specimen will be processed on the molecular level via MALDI-TOF mass-spectrometry in order to correlate molecular changes to local changes in biomechanical properties. Furthermore, this experimental setup allows studying the effects of drug induced cellular remodeling processes at close proximity hence allowing to shade light on specific molecular processes during fibrotic alterations of tissue.

References [1] Huwart L, NMR Biomed 2006: 19(2):173-9; [2] Yin M, Clin Gastroenterol Hepatol 2007:5(10):1207-13; [3] Klatt D, Phys Med Bio 2007:52(24):7281-94; [4] Muthupillai, R, et al, Science, 1995. 269(5232) p. 1854 ; [5] Sinkus R, Tanter M, et al. Magn Reson Imaging 2005 : 23:159 ; [6] Bedossa P et al. Hepatology 1996 : 24(2):289-93.