MRI of liver fibrosis with a fibrin-specific probe

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Target audience: Researchers and physicians interested in MRI tools for detection of liver fibrosis.

Purpose: Liver fibrosis is characterized by excessive accumulation of extracellular matrix proteins in response to repeat liver injury. Current understanding of fibrogenesis suggests that initial steps of tissue repair include extravascular clot formation. Accordingly, it has been hypothesized that accumulation of fibrin and fibronectin precede the increase of other connective tissue proteins and could serve as an early marker of fibrosing liver damage [1]. Fibrin deposition in the liver is anticipated after acute injury due to vascular leakage caused by endothelial damage. However, an immunostaining-based study by Neubauer at el [1] revealed abnormal fibrin accumulation not only after short-term injury, but also in chronically injured tissue. EP-2104R is a gadolinium-based contrast agent that specifically targets fibrin and has been previously reported for detection of thrombus [2, 3]. In this study we investigate whether MRI with EP-2014R could be used to detect abnormal fibrin deposition in vivo in a rat model of moderate liver fibrosis.

Methods: *Animal model:* Liver fibrosis was induced in male Wistar rats with weekly intraperitoneal injections of 100 mg/kg diethylnitrosamine (DEN). Control animals received PBS. Long-term injury was studied in six DEN-treated and five control animals, all of which underwent four weeks of treatment and were imaged one week after the last injection (delayed injury phase) to avoid acute DEN effects. In addition, in order to study the effect of acute injury, six rats were treated for five weeks and imaged 24 hours after the fifth DEN dose (acute injury phase). *Imaging:* Imaging was performed on a human whole-body 1.5T system (Avanto, Siemens) with a custom-built transmit-receive coil. Animals were anesthetized with isoflurane (1–2%) for the duration of the experiment. A series of T1-weighted images (respiratory-triggered 3D VIBE sequence; TR/TE=6.28/1.84ms, FOV = 93x120 mm², matrix=150×192, 36 0.6-mm slices, 1 average, and acquisition time = 50-60 sec) were obtained prior to and following intravenous injection of 5 µmol/kg EP-2104R. Imaging was repeated continuously for 40 minutes following contrast delivery. Mean signal intensity (SI) of liver parenchyma was estimated at baseline (SI_{pre}) and at various time points after EP-2104R injection (SI_{post}). Percent change in post-contrast liver SI relative to baseline was calculated as: $\%\Delta SI=100*(SI_{post}-SI_{pre})/SI_{pre}$. The area under the time-signal intensity curve (AUC) after 20 minutes of imaging was also calculated. Unpaired Student's t-Test was used for statistical analysis where $p < 0.05$ was considered as significant. *Tissue analysis:* Rats were euthanized at the end of the imaging session. Immunohistochemistry was performed to detect fibrin. The degree of liver fibrosis for each animal was accessed by Sirius red staining, which was quantified using ImageJ.

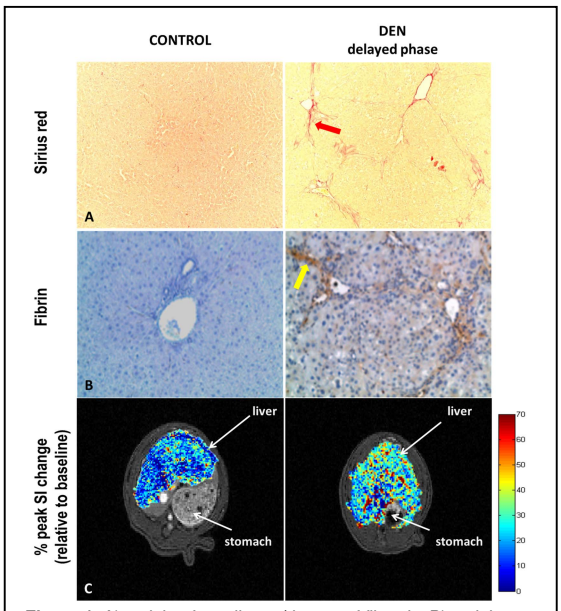

Figure 1. A) staining for collagen/degree of fibrosis; B) staining for fibrin; C) VIBE MRI of rat liver during peak signal enhancement of EP-2104R. False color overlay is pre-injection image subtracted from post-injection image.

Results: Control animals showed no disease, while DEN-treated animals exhibited staining patterns (red arrow) representative of moderate fibrosis (ISHAK score 3-4) (Fig. 1A). Amount of fibrosis did not differ between the delayed phase and the acute phase cohorts. Fibrin-positive staining (yellow arrow) was observed in DEN-treated animals, but not in controls (Fig. 1B). Fig. 1C illustrates the magnitude of peak signal enhancement characteristic of control and DEN-treated animals (delayed injury phase). In all animals EP-2014R resulted in marked increase in liver SI immediately after injection, followed by rapid wash-out and signal plateau close to baseline as early as 5-10 minutes post contrast. Control and fibrotic animals exhibited different mean AUC values (control: 101±6; delayed phase DEN: 145±19; acute phase DEN: 171±41), but the differences did not reach statistical significance (Fig. 2A). Fig. 2B shows %SI change relative to baseline during peak enhancement. Fibrotic animals imaged in the delayed injury phase demonstrated 35% higher change in peak liver SI compared to controls (control: 17.1±0.2%, delayed phase DEN: 23.1±2.0%, p = 0.031). The acute phase cohort exhibited even larger peak %ΔSI: 68% higher than controls (controls: 17.1±0.2%, acute phase DEN: 28.7±3.2%, p=0.003) and 24% higher than the other DEN-treated cohort (delayed phase DEN: 23.1±2.0%, acute phase DEN: 28.7±3.2%, p=0.008).

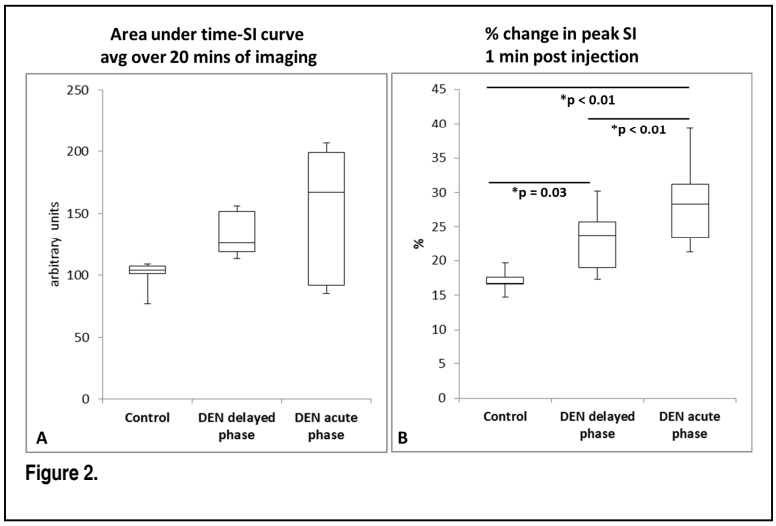

Figure 2.

Conclusion: AUC and peak %ΔSI following EP-2104 injection were higher in fibrotic animals than in controls. This effect is likely a result of higher buildup of EP-2104R in DEN-treated animals due to increased fibrin levels in fibrotic livers. Our ongoing work includes development of ex vivo tissue analysis methods for fibrin quantification to determine a numerical relationship between liver fibrin and EP-2104R signal enhancement. Fibrotic livers imaged after acute insult exhibited higher AUC and peak %ΔSI compared to DEN-treated animals imaged during the delayed injury phase even though the two cohorts had similar levels of fibrosis. This is likely a consequence of both increased fibrin accumulation and inflammation caused by the acute insult. We are performing in vivo studies with a non-binding version of EP-2104R and histological quantification of inflammation to determine the origin of the stronger MRI signal in acutely injured animals. In conclusion, our results indicate that MRI with a fibrin specific probe could be used to characterize earlier stages of liver fibrosis as the method can differentiate moderate fibrosis from healthy tissue and is also sensitive to acute effects of liver injury.

References: [1] Neubauer et al; Gastroenterology 1995;108:1124; [2] Overoye-Chan et al; JACS *2008*; 130(18): 6025; [3] Vymazal et al; Invest Radiol 2009; 44: 697