

MRI of Liver Fibrosis by Fibrin-Fibronectin Targeted Contrast Agent

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Introduction

Liver fibrosis, associated with chronic liver injury, including hepatitis and alcohol intoxication, can progress to cirrhosis and hepatocellular carcinoma. It is characterized by an increased amount of extracellular matrix consisting of fibrin-forming collagens and matrix glycoconjugates such as fibronectin¹. The fibrin-fibronectin complexes in fibrotic liver, resulted from cross-linkage between fibrin/fibrinogen and fibronectin², may serve as a specific molecular target for contrast-enhanced MRI. Incorporation of fibrin-fibronectin targeted peptides to gadolinium (Gd) chelates^{3,4} may result in a sensitive and specific contrast agent for molecular MR imaging of liver fibrosis. This preliminary study aimed to demonstrate that a fibrin-fibronectin targeted Gd contrast agent provided distinct contrast enhancement in fibrotic liver, as compared with a non-targeted Gd contrast agent, in an experimental model.

Methods

Animal Procedure: Liver fibrosis was induced in male C57 mice (25-28 g; $N = 4$) by subcutaneous injection of 1:3 mixture of CCl_4 in olive oil at a dose of $4 \mu\text{L/g}$ of body weight twice a week for 6 weeks⁵. Femoral vein catheterization was performed with a 1-m long tube connected to a 30-gauge needle under anesthetization using 1.5-2% isoflurane/air.

Contrast Agent: A CLT1 (CGLIIQKNEC) peptide targeted nanoglobular contrast agent (Gd-P) specific to fibrin-fibronectin complexes^{3,4} was intravenously injected at a dose of 0.03 mmol Gd/kg of body weight ($N = 2$). A non-targeted nanoglobular contrast agent (Gd-C) was injected at a dose of 0.03 mmol Gd/kg of body weight ($N = 2$) for comparison. The nanoglobule was a generation 3 (G3) poly-L-lysine dendrimer with an octa(3-aminopropyl)silsesquioxane (OAS) cubic core, exhibiting a relatively compact and rigid globular structure⁶. The G3 nanoglobule was synthesized in a good yield and purity using solution phase peptide chemistry. CLT1 peptide was synthesized by solid phase peptide chemistry and conjugated to the nanoglobular contrast agent via click chemistry. Compounds were characterized by mass spectrometry, and the Gd content was determined by inductively coupled plasma (ICP)-optical emission spectroscopy. Approximately 3 peptides and 43 Gd-DOTA chelates on average were conjugated onto the surface of the nanoglobular dendrimer (total 64 amine groups).

MRI: All MRI experiments were performed on a 7 Tesla Bruker MRI scanner using a 38-mm quadrature RF resonator. Each animal was placed in supine position with the abdomen fixed with adhesive tape to reduce respiratory movement and was anesthetized with 1-1.5% isoflurane/air via inhalation for maintenance. Body temperature was maintained at about 36.5°C by circulating warm water in a heating pad with respiratory monitoring. A reference phantom containing phosphate-buffered saline (PBS) was placed next to each animal. Contrast-enhanced MRI was performed before and every 5 min after contrast agent administration for 60 min, using axial T1-weighted 2D spin-echo (SE) sequence with TR = 200 ms, TE = 8 ms, FOV = $30 \times 30 \text{ mm}^2$, acquisition matrix = 128×128 , slice thickness = 1 mm, slice gap = 1 mm, number of slices = 3, NEX = 6, and scan time = 2 min.

Data Analysis: A large region of interest (ROI) was drawn in homogeneous region of liver parenchyma, with care avoiding inclusion of major vascular structure. In addition, a circular ROI was drawn in the reference phantom. Normalized liver signal intensity was calculated as the ratio of signal intensity of liver to that of the phantom, and was averaged over slices in each animal.

Histology: Animals were sacrificed at 1 day after MRI. One normal animal was sacrificed as control. The livers were embedded in paraffin, sectioned, and examined by light microscopy after standard hematoxylin-eosin (H&E) staining.

Results and Discussions

Fig. 1 shows the representative T1-weighted SE images of fibrotic liver before, 5 and 60 min after injection of Gd-C and Gd-P. Fig. 2 shows the normalized liver signal intensity time curves with Gd-C and Gd-P injection. Gd-P consistently showed higher contrast enhancement than Gd-C in fibrotic liver up to 60 min postinjection. Note that the first-pass effect of the contrast agents was not studied. The contrast enhancement of Gd-P was higher than that of Gd-C at 5 min postinjection, suggesting the effective targeting capability of Gd-P. Furthermore, contrast enhancement by Gd-P persisted during the period of 60 min postinjection, whereas enhancement by Gd-C decreased and vanished rapidly after the maximum enhancement. Fig. 3 shows the typical H&E staining of normal liver and liver after 6-week CCl_4 intoxication. Compared with normal liver, the liver with 6-week CCl_4 insult showed obvious collagen depositions throughout the liver, confirming liver fibrosis in the animals studied.

Conclusion

The preliminary results suggested that the fibrin-fibronectin specific contrast agent, Gd-P, could specifically bind to fibrin-fibronectin complexes in fibrotic liver, leading to strong and prolonged contrast enhancement. Such target-specific contrast-enhanced molecular MRI may be valuable in detecting and characterizing liver fibrosis at early phase. Note that the results were limited by the small sample size, and lack of normal animals with Gd-P injection as control. Study with larger sample size and immunohistochemical verification of presence of fibrin-fibronectin complexes in fibrotic liver is currently underway to fortify these preliminary results.

References [1] Friedman SL et al. *N Engl J Med* 1993;328:1828-1835. [2] Neubauer K et al. *Gastroenterology* 1995;108:1124-1135. [3] Ye F et al. *Bioconjug Chem* 2008;19:2300-2303. [4] Pilch J et al. *Proc Natl Acad Sci* 2006;103:2800-2804. [5] Constandinou CN et al. *Methods Mol Med* 2005;117:237-250. [6] Kaneshiro T et al. *Biomacromolecules* 2008;9:2742-2748.

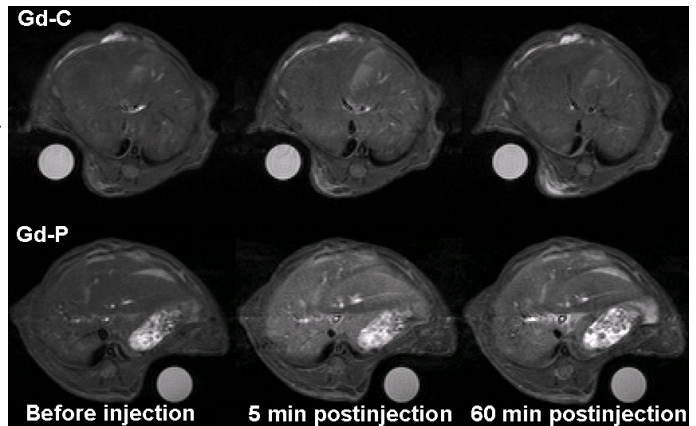


Fig. 1. T1-weighted spin-echo images of fibrotic liver before, 5 and 60 min after injection of Gd-P (0.03 mmol Gd/kg) and Gd-C (0.03 mmol Gd/kg).

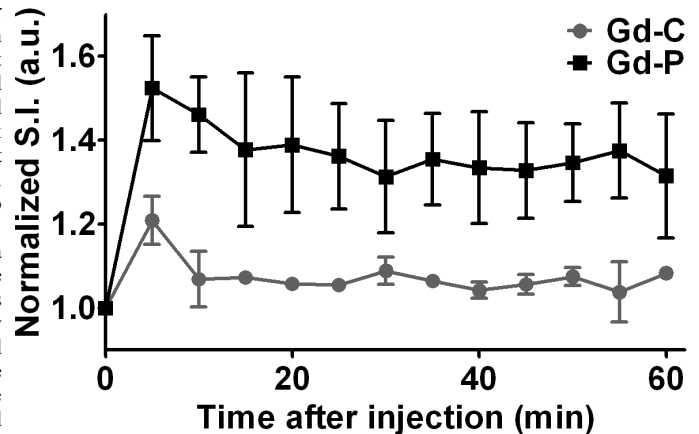


Fig. 2. Normalized liver signal intensity (S.I.) time curves with Gd-C ($N = 2$) and Gd-P ($N = 2$) injection. Error bars represent standard deviation.

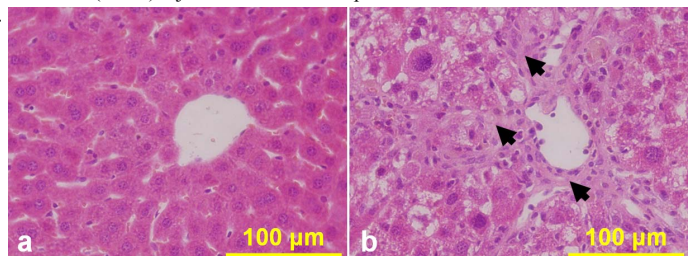


Fig. 3. Typical H&E staining of normal liver (a) and liver after 6-week CCl_4 insult (b). Black arrows indicate collagen depositions in the insulted liver.