MOLECULAR MRI OF LIVER FIBROSIS BY FIBRIN-FIBRONECTIN TARGETED CONTRAST AGENT 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 lead to its early interventions and treatments, thus prevent its progression to cirrhosis[2]. Recently, specific binding of a cyclic decapeptide CGLIIQKNEC (CLT1) to the fibronectin-fibrin complexes, formed by clotting of fibronectin isoforms including oncofetal fibronectin[3] and an alternatively spliced form of fibronectin[4], has been observed in the extracellular matrix of different tumors and tissue lesions[5]. Liver fibrosis is characterized by an increased amount of extracellular matrix consisting of fibril-forming collagens, and matrix glycoconjugates such as fibronectin[6]. Recently, fibronectin isoforms has been identified as a biomarker for liver fibrosis[7]. Fibrin-fibronectin complexes exist in fibrotic liver due to the cross-linkage between fibrin/fibrinogen and fibronectin[8]; therefore, they may serve as a specific molecular target for contrast-enhanced MRI. In this study, we aim to investigate the feasibility of detecting early liver fibrosis using CLT1 peptide-targeted nanoglobular contrast agent with MRI in an experimental mouse model.

METHODS

Synthesis and MR Characterization of Contrast Agents: CLT1 peptide-targeted nanoglobular contrast agent (Gd-P) and control non-targeted KAREC peptide nanoglobular contrast agent (Gd-CP) were synthesized as described previously[9]. Note that KAREC peptide shows no binding to fibronectin-fibrin complexes in tumors[5]. ***Animal Preparation:*** Male adult C57BL/6N mice (22-25g; N=36) were prepared. Liver fibrosis was induced in fibrosis group (N=24) by subcutaneous injection of 1:3 mixture of CCl_4 in olive oil at a dose of 4μL/g twice a week for 8 weeks[10]. DCE-MRI was performed in the CCl_4-insulted animals at 4 and 8 weeks after the start of CCl_4 administration. Normal intact animal group (N=12) served as controls. ***MRI:*** All MRI experiments were performed on a 7T Bruker MRI scanner using a 38-mm quadrature RF coil, with isoflurane anaesthesia, animal warming with 37ºC by circulating water pad, and respiratory monitoring. A saline phantom was placed next to each animal. Preinjection T_1 values were measured with a series of SE images with varying TRs=125,250,500,1000,2000,4000ms, TE=8ms. DCE-MRI of mouse liver was then performed with T_1-weighted 2D FLASH sequence for 1 hour using TR/TE=50/2.5ms, FA=80º, spatial resolution=0.23×0.23×2.0mm^3, NEX=4 and 20s temporal resolution. For each imaging session, 0.1mL of contrast agent (0.03mmolGd/kg) was injected intravenously via femoral vein catheterization at 3mL/min at 6min after the start of dynamic scan. ***Data Analysis:*** Liver signal intensity was first normalized to that of phantom and was averaged over three slices in each animal. Assuming negligible T_2 effects from the injected contrast agents due to the short TE and low dose used, peak ΔR_1 maps were computed on a pixel-by-pixel basis[11] as $S_{pre}/S(t) \approx (1-\exp(TR \cdot R_{1pre}))/(1-\exp(TR \cdot R_1(t)))$, where S_{pre} is the average intensity of 15 preinjection images and S(t) is the intensity of postinjection image with maximum contrast enhancement. Similarly, steady-state ΔR_1 maps were obtained using S(t) as the average intensity of 90 postinjection images at the end of the dynamic scan. To estimate liver ΔR_1, a large ROI was manually drawn in a homogeneous liver region. One-way ANOVA with Tukey's multiple comparison tests were employed to compare differences in ΔR_1 values in different groups. ***Histology:*** Animals were sacrificed after MR examinations. Immunohistochemistry was done to detect fibronectin using primary antifibronectin antibody[9].

RESULTS

Fig. 1 illustrates the liver image enhancements typically observed during Gd-P and Gd-CP injections for mice after 4-week CCl_4 insult. Similar enhancement patterns were observed in mice after 8-week CCl_4 insult and normal control mice. Fig. 2 shows the *in vivo* measurements of peak and steady-state ΔR_1 for all animals studied. Fig. 3 shows the typical fibronectin staining of normal liver and livers at 4 weeks and 8 weeks after CCl_4 insult. The insulted livers showed increased amount of fibronectin in the extracellular space as revealed by brown deposits (Figs. 3(b) and (c)), which were consistent with those observed in previous hepatic fibrosis immunohistochemical study[8].

DISCUSSIONS

For all the animals studied, the stronger contrast enhancement of Gd-P compared to Gd-CP in liver at postinjection steady-state indicated the specific binding of Gd-P to hepatic fibronectin. Note that the existence of fibronectin in normal liver due to its function of producing plasma fibronectin[7,8]. In contrast, non-targeting effects of Gd-CP were confirmed by its vanishing enhancement after the maximum enhancement for all animals. Both the peak and steady-state ΔR_1 values after Gd-P were higher in fibrotic livers than in normal livers, likely as a result of the increased amount of accumulated Gd-P due to increased fibronectin in fibrotic livers. From both peak and steady-state ΔR_1 analysis, fibrotic livers can be readily distinguished from normal livers following injection of Gd-P as early as week 4 after CCl_4 insult.

CONCLUSIONS

In this study, we investigated the feasibility of Gd-P for early detection of liver fibrosis through molecular imaging of fibronectin at 7T. Considerable contrast enhancements were observed and characterized in normal and fibrotic livers using Gd-P at a relative low dose as compared to Gd-CP. Differential enhancements between normal and fibrotic livers were only found for Gd-P. Our results indicate that Gd-P could be used as a fibrin-fibronectin specific MR contrast agent to detect and characterize liver fibrosis at early phase.

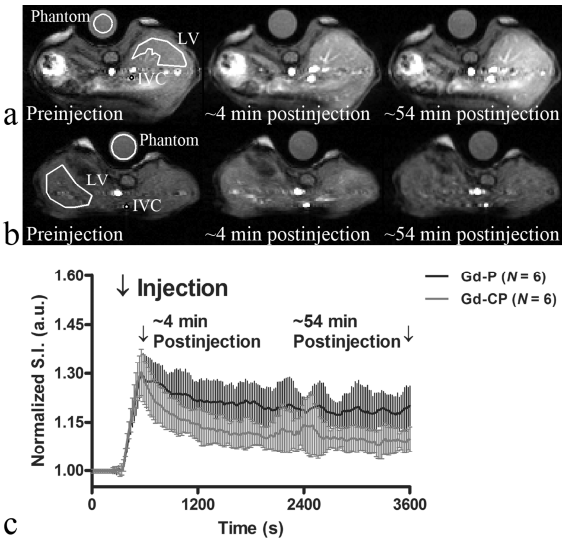

FIG. 1. Corresponding images from the mouse liver after 4-week CCl_4 insult. DCE images for (**a**) CLT1 peptide-targeted nanoglobular contrast agent (Gd-P) and (**b**) control non-targeted KAREC peptide nanoglobular contrast agent (Gd-CP) at 0.03mmolGd/kg. (**c**) Normalized liver signal intensity time curves for animals after 4-week CCl_4 insult.

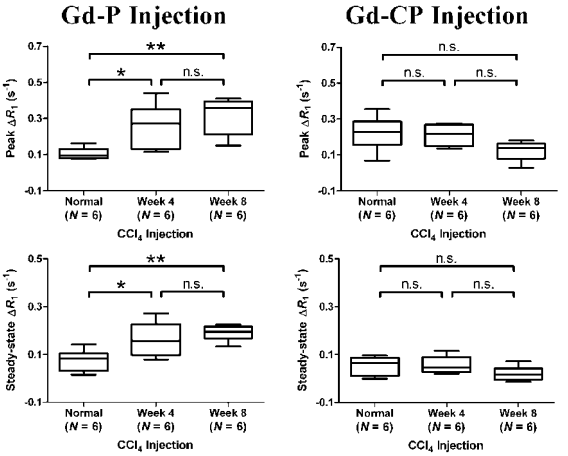

FIG. 2. *In vivo* measurements of peak and steady-state ΔR_1 for all animals studied. One-way ANOVA was performed with * for p<0.05, ** for p<0.01 and n.s. for insignificance.

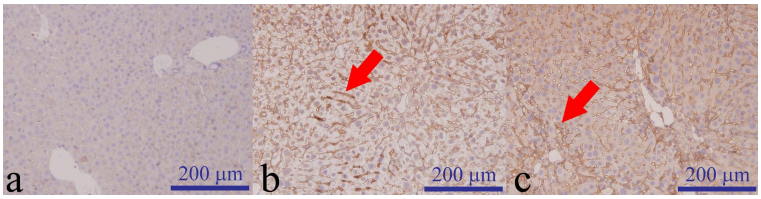

FIG. 3. Fibronectin staining (200×) of (**a**) normal liver, and livers subjected to (**b**) 4-week and (**c**) 8-week CCl_4 twice-weekly administration. Fibronectin (red arrows) was observed in the insulted livers as brown deposits.

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