Molecular Imaging of Collagenous Scar Tissue in Chronic Myocardial Infarction Using a Targeted MR Contrast Agent

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Introduction: In normal tissue collagen forms an external scaffold to support cellular structure and function. In cardiac tissue, collagen, of which ~85% is type I, forms a dynamic construct that not only supports and aligns myocytes into an energetically favorable architecture, but also transmits active and passive forces throughout its structure [1, 2]. In response to tissue injury, collagen synthesis is upregulated during fibrosis and scar formation. Hepatic fibrosis, a common result of chronic injury from insults such as viral hepatitis, alcohol abuse or drugs occurs several months after the initial insult. Pulmonary fibrosis manifesting from asbestosis, chronic smoking, or other pathogens causes significant changes in the extracelluar matrix and leads to irreversible distortion of the lung's architecture [3]. Molecular imaging of collagen would be valuable for the detection and monitoring of such diseases. A clinically relevant animal model for testing a collagen-targeted molecular imaging agent is post-infarct myocardial scar tissue. After myocardial infarction (MI), wound healing results in the generation of fibrotic scar tissue. We evaluated a novel gadolinium-based collagen-targeted contrast agent (EP3533, EPIX Pharmaceuticals, MA) for improved scar imaging in a mouse model of chronic MI.

Methods: A collagen-specific contrast agent, EP3533, was designed by appending Gd-DTPA moieties for positive contrast imaging to a collagen-specific peptide for molecular targeting. EP3533 binds reversibly to mouse type I collagen (K_d = 1.8 μM) with a high stoichiometry of binding (N>7 equivalent binding sites). MI was induced in six C57BL/6 mice by a 60 minute occlusion of the left anterior descending coronary artery followed by reperfusion. MRI studies were performed 40 – 45 days later, after scar formation was fully complete[4]. Indwelling tail vein lines were used for I.V. injection of contrast agents during imaging. Six mice were injected with conventional non-targeted Gd-DTPA, 0.2 mmol/kg (Magnevist, Berlex) and in a follow up study two days later EP3533 (0.025 mmol/kg) was injected during imaging. Doses were chosen to give equivalent relaxivity of both agents at 4.7T. Imaging was performed using a 4.7T MRI system (Varian, CA), and included ECG-gated localizer scans, cine MRI, and inversion-recovery (IR) imaging before and serially (every 5 minutes) after contrast agent injection. Signal to noise ratios were calculated as a function of time for regions of interest in the infarct zone, normal zone, and blood pool (LV chamber). After all imaging studies were completed, three hearts were explanted and sectioned in 10μm thick intervals from base to apex and, then stained with picrosirius red to compare areas of MR enhancement with areas of collageneous scar (as determined by histology). The remaining three hearts were grossly sectioned into scar versus non-scar at 50 minutes post-injection and gadolinium content (nmol Gd/g heart) was determined by inductively coupled plasma mass spectrometry (ICP-MS).

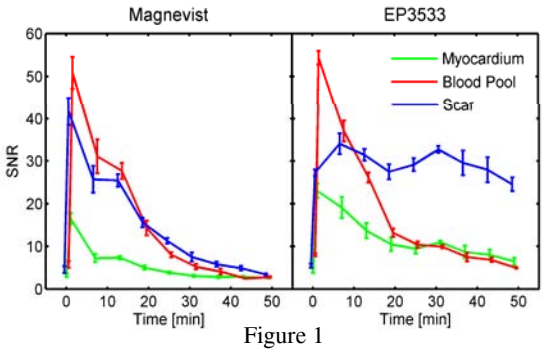

Figure 1

Results: Serial IR imaging after Gd-DTPA injection showed strong early enhancement of the blood pool and scar, followed by washout over a period of 30 minutes (left panel Fig. 1). Mild early enhancement of normal myocardium was also seen. EP3533 also showed early enhancement of the blood pool with washout beginning 10 minutes after injection (right panel Fig. 1). Strong enhancement of scar peaked approximately 10 minutes after injection and persisted for at least 50 minutes. Example images correlating histology measurements of collagenous scar (red stain) with IR imaging of EP3533 and Magnevist are shown in Fig. 2. The concentration of Gd^{+3} in scar tissue versus normal myocardium tended to be higher at 76.5±21.4 and 51.7±19.2, respectively ($p<0.13$).

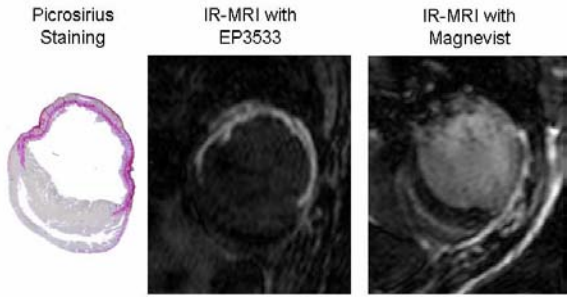

Figure 2

Conclusions: Molecular imaging of collagen provides high contrast between fibrotic scar and normal myocardium or blood in a mouse model of chronic, fully healed MI. Imaging 30 – 40 minutes after injection of EP3533 may be optimal because unbound agent has washed out of the blood and normal myocardium, while targeted agent bound to collagen continues to strongly enhance the scar.

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