Title: “Myocardium”
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Molecular imaging of the myocardium is frequently performed with nuclear imaging techniques (SPECT and PET). These techniques have extremely high sensitivity, and the radiotracers used are small and thus easily able to enter the interstitial space of the myocardium. Over the last several years, however, molecular MRI techniques to image the myocardium have been developed and are now extremely competitive with the established nuclear imaging techniques.

Four broad strategies underlie current molecular MRI techniques in the myocardium: 1) The imaging of highly expressed targets with small low relaxivity Gd chelates, 2) The use of targeted iron-oxide nanoparticles to image more sparsely expressed targets, 3) The use of MR-detectable nanoparticle agents to image inflammatory cells in the myocardium, and 4) Imaging targets on the surface of the capillary endothelium in the myocardium.

Large amounts of collagen are found in the myocardium. A small Gd chelate, similar to those used clinically, can thus be used to image this target. A 15 amino acid peptide, that binds avidly to type 1 collagen, was thus conjugated to 3 Gd-DOTA chelates to form a specific collagen binding agent (1). The probe has been used to characterize collagen content in the myocardium in three scenarios. In acute ischemic injury the agent can be used to image perfusion defects. This was robustly shown in a swine model of non-occlusive coronary artery stenosis (2). Chronic infarction is characterized by the replacement of cardiomyocytes with a dense collagen rich scar. Conventional Gd chelates display late enhancement in a chronic infarct, but this is a non-specific pharmacokinetic signature and is seen in acute injury as well. Specific assessment of infarct collagen content is thus more robustly determined through the use of a collagen-targeted probe. This was robustly demonstrated in a mouse model of chronic infarction, where prolonged Gd retention in the infarct (greater than one hour after injection) was seen with the collagen-targeted probe but not with the scrambled control probe (3). The collagen targeted probe has also been used to image the diffuse myocardial fibrosis that accompanies left ventricular hypertrophy. T1 maps of probe uptake were acquired in mice 4 weeks after aortic banding.

Nuclear DNA is another highly abundant target in the myocardium. In healthy myocardium, however, the intact cell membrane prevents conventional Gd chelates from entering the cell and gaining access to this intracellular target. Necrotic cells with injured membranes, however, pose no such barrier and allow their DNA to be imaged. A DNA-targeted Gd chelate has thus been developed as an imaging marker of acute necrosis. The agent consists of the vital (DNA-binding) fluorochrome Thiazole Orange (TO) conjugated to Gd-DTPA (4). The specificity of the agent for acutely necrotic cells was confirmed in a mouse model of myocardial infarction. Uptake of the agent was seen within two hours of injury but was no longer seen when injected more than 72 hours after infarction, by which time the necrotic cells and DNA had been cleared by infiltrating monocytes.

Iron-oxide nanoparticles are ideally suited to imaging sparsely expressed targets in the myocardium. They are small (30-50 nm), inert and have very high magnetic relaxivities. These agents are able to cross capillary membranes, access the interstitial space, avoid non-specific uptake, and bind selectively to the

target of interest. The largest experience to date with these nanoparticles is with the apoptosis sensing agent AnxCLIO-Cy5.5. This has been shown to bind to apoptotic cardiomyocytes in both ischemia reperfusion and heart failure (5,6). The effective or "per dose" sensitivity of AnxCLIO-Cy5.5 is similar to that of radiolabeled annexin V. This is because the amount of AnxCLIO-Cy5.5 injected is limited by the iron content of the probe and not by the radiation dose. Consequently a single dose of AnxCLIO-Cy5.5 (3mg Fe/kg) can contain 3 orders of magnitude more annexin V than a single dose of ^{99}TC-annexin. A clinical dose of AnxCLIO-Cy5.5 and ^{99}TC-annexin thus show comparable sensitivity, and both have been able to image very low levels of cardiomyocyte apoptosis in heart failure (6).

One of the limitations of annexin, and hence AnxCLIO-Cy5.5, is that it binds to both apoptotic and necrotic cells. A dual contrast molecular MRI strategy was thus developed to resolve apoptosis and necrosis in vivo (7). AnxCLIO-Cy5.5 was injected at the onset of reperfusion and its uptake in the myocardium was imaged within 4 hours. To differentiate AnxCLIO-Cy5.5 uptake by apoptotic or necrotic myocardium, Gd-DTPA was then injected and delayed enhancement imaging was performed. Areas of myocardium with AnxCLIO-Cy5.5 uptake and delayed Gd enhancement could thus be correctly classified as necrotic rather than apoptotic (7). It should be noted that this strategy is suited to imaging at high field strengths (9.4 T) since the R1 effects of iron-oxide, which are considerable at lower field strengths, can be easily negated at 9.4 T.

Myocardial injury is frequently followed within 24 hours by a robust inflammatory infiltrate. Iron-oxide nanoparticles, liposomes and other nano-sized imaging agents are robustly recognized by these inflammatory cells. Targeted imaging with nano-sized agents must thus be performed well within 24 hours of injury, before the infiltrate develops, or 2-3 weeks later once it resolves. The uptake of these agents by macrophages infiltrating the myocardium, however, can be exploited to image the presence and severity of inflammation in the myocardium. Iron-oxide nanoparticles, for instance, have been used to image inflammation several days after infarction and in transplant rejection (8).

The myocardium is a highly vascularized tissue and the capillary endothelium thus presents a large target. Following ischemia, adhesion molecules (VCAM-1) and selectins are expressed on the capillary endothelium and could potentially be imaged with MRI. Since these targets are expressed on the endothelial surface the use of large constructs such as liposomes and microparticles could be considered. Non-specific extravasation and uptake by inflammatory cells, however, will need to be carefully watched for. The endothelial cells involved in angiogenesis in the healing infarct have recently been imaged with a CD13-targeted nanoparticle construct (9).

In summary, molecular MRI of the myocardium has evolved rapidly in the last few years and now provides a platform to image many of the central processes in cardiovascular pathophysiology. The technique is flexible and, when combined with the unsurpassed capability of MRI to image the myocardium, constitutes and extremely powerful and comprehensive imaging approach.

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