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Targeted imaging of myocardial damage

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SUMMARY

Molecular imaging agents can be targeted to a specific receptor or protein on the cardiomyocyte surface, or to enzymes released into the interstitial space, such as cathepsins, matrix metalloproteinases and myeloperoxidase. Molecular imaging of the myocardium, however, requires the imaging agent to be small, sensitive (nanomolar levels or better), and able to gain access to the interstitial space. Several novel agents that fulfill these criteria have been used for targeted molecular imaging applications in the myocardium. Magnetic resonance, fluorescence, and single-photon emission CT have been used to image the molecular signals generated by these agents. The use of targeted imaging agents in the myocardium has the potential to provide valuable insights into the pathophysiology of myocardial injury and to facilitate the development of novel therapeutic strategies.

Keywords

fluorescence imaging; molecular imaging; MRI; myocardium; SPECT

INTRODUCTION

This brief review focuses on research relating to targeted molecular imaging in the myocardium, a rapidly growing field with immediate preclinical applications and near-future impact in clinical cardiology.^{1–3} The performance of targeted molecular imaging in the myocardium is dependent on the ability of the imaging agent to penetrate the capillary membrane and enter the interstitial space of the myocardium. Thereafter, the agent needs to bind specifically to intended molecular targets, often expressed in the low-nanomolar range, while avoiding nonspecific uptake in the complex milieu of the interstitial space. These challenges impose important size, inertness, and sensitivity constraints on potential imaging agents.¹

The attributes of the technique used to image the targeted agent are also an extremely important consideration. Table 1 summarizes the strengths and limitations of nuclear, magnetic resonance, and fluorescence imaging techniques for targeted molecular imaging in the myocardium. The use of serological markers such as troponin, brain natriuretic peptide, and other novel genomic and proteomic readouts has the potential to provide highly complementary information to molecular imaging. Molecular imaging of a biological process in the

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myocardium, however, allows the spatial distribution and local impact of the process to be determined, independent of conditions elsewhere in the heart or in the body.

NUCLEAR IMAGING

Nuclear imaging techniques have been the most extensively used to date for molecular imaging of the myocardium and have several advantages and disadvantages.² Although these techniques are extremely sensitive (picomolar levels of injected tracers can be detected), their relatively low spatial resolution (millimeter to centimeter) might not allow the transmural distribution of a targeted imaging agent to be adequately resolved.⁴ Dual-isotope single-photon emission CT (SPECT) imaging is capable of resolving two separate molecular signals simultaneously,^{5,6} which is a highly advantageous ability; however, injected nuclear agents are always radioactive and thus not capable of being activated by biological processes *in vivo*, unlike agents for optical imaging and MRI.

The imaging of antimyosin antibodies with nuclear techniques was one of the earliest applications of targeted imaging in the myocardium. Myosin is an intracellular protein, so the probe can bind to the target only in the presence of cell membrane disruption and exposure of the intracellular protein. Initial testing of the probe was performed in infarcted dogs injected with indium-111-labeled (¹¹¹In) antimyosin antibodies.⁷ Translation into the clinical setting was then successfully performed using SPECT imaging of technetium-99m-labeled (^{99m}Tc) antimyosin Fab fragments in 30 patients with acute myocardial infarction.⁸

SPECT imaging has also been used to image cardiomyocyte apoptosis/cell death in patients.^{9,10} Annexin-V is a calcium (Ca²⁺)-dependent phospholipid-binding protein that binds specifically to phosphatidylserine, a phospholipid expressed on the outer cell membrane of apoptotic cells. ^{99m}Tc annexin-V was injected into patients with acute myocardial infarction 2 h after percutaneous revascularization.⁹ Increased annexin-V uptake was seen in the infarct area on SPECT imaging, and isotope uptake colocalized with perfusion deficits detected 6–8 weeks after hospital discharge (Figure 1A,B). Imaging with ^{99m}Tc annexin-V has also been used to assess apoptosis/cell death in the myocardium of 18 patients with potential transplant rejection, five of whom showed uptake of the probe.¹⁰ Transplant rejection in these five patients was confirmed histologically, and positive staining for caspase-3, the key enzyme in the apoptotic cascade, confirmed the presence of cardiomyocyte apoptosis. The ability to image cardiomyocyte apoptosis (annexin-V) and necrosis (antimyosin) *in vivo* with these techniques could enable the early diagnosis of transplant rejection to be made without myocardial biopsies and guide therapeutic strategies in acute ischemic syndromes, myocarditis, transplant rejection, and heart failure.

The transglutaminase factor XIII is involved in the regulation of leukocyte recruitment, angiogenesis, and scar formation in healing infarcts.¹¹ Factor XIII activity in healing myocardial infarcts in mice was successfully visualized with use of a ¹¹¹In specific substrate peptide for factor XIII (Figure 1C). Factor XIII imaging in patients could thus conceivably allow infarcts that are at risk for poor healing to be identified and could also be used to assess the potential of local intramyocardial factor XIII supplementation.

Matrix metalloproteinases (MMPs) and angiogenesis play important parts in myocardial infarct healing. A ^{99m}Tc compound that binds to the catalytic domain of the MMP enzyme has been used in mice to image MMP levels at different time points following coronary ligation (Figure 1D,E).⁶ Angiogenic vessels express the α₅β₃ integrin on their surface, and angiogenesis in the myocardium of infarcted dogs has thus been imaged with an ¹¹¹In quinolone, targeted to the α₅β₃ (Figure 1F,G).⁵ Peak uptake of the agent was detected 1 week after myocardial infarction, during the period of greatest angiogenesis. Imaging of MMPs and angiogenesis could allow these processes to be monitored locally in the healing infarct *in vivo* and thus facilitate the

development of strategies to modulate their activity, maximize local healing, and minimize left ventricular remodeling.

MRI

Experience with molecular MRI in the myocardium is less extensive than that with nuclear imaging agents, but the characteristics of MRI make it highly suited to myocardial imaging.¹² MRI is able to assess a range of anatomical, physiological, mechanical, metabolic and hemodynamic parameters in the myocardium (Table 1): myocardial function can be assessed with cine and strain-based techniques, metabolism with spectroscopy, perfusion with first-pass imaging of gadolinium, and viability with delayed-enhancement imaging. The spatial resolution of MRI is excellent, and it does not involve exposure to ionizing radiation, a particularly important consideration for molecular imaging screening studies aimed at the early detection of disease in relatively young people.¹³ The principal challenge of applying molecular MRI, however, lies in its lower sensitivity for the detection of conventional imaging agents.¹ Novel gadolinium constructs (both small molecules and supramolecular structures) and iron-oxide-based magnetic nanoparticles (MNPs) have thus been developed.¹ Second-generation and third-generation MNPs are typically 20–50 nm in size, long circulating, and detectable at nanomolar sensitivity with T2-weighted pulse sequences. These characteristics make them particularly well suited for targeted imaging in the myocardium.^{14,15}

In one of the first examples of targeted molecular MRI, based on earlier nuclear imaging experience, antimyosin antibodies were labeled with MNPs to image necrotic cardiomyocytes during acute myocardial infarction in rats.¹⁶ Accumulation of antimyosin-labeled MNPs in the infarct zone was shown by *ex vivo* MRI (Figure 2A), whereas no accumulation of unlabeled MNPs was seen.¹⁶ The ability to image cardiomyocyte necrosis with high-resolution MRI could provide novel insights into the spatial distribution and functional significance of cardiac injury in a variety of important conditions, including myocardial infarction, myocarditis, and transplant rejection.

Cardiomyocyte apoptosis plays an important role in ischemia, reperfusion injury, and heart failure and has been imaged with MRI *in vivo* using AnxCLIO-Cy5.5, an annexin-labeled magneto-fluorescent nanoparticle.^{17,18} The accumulation of AnxCLIO-Cy5.5 in a mouse model of transient coronary ligation was detected with MRI, *in vivo*, in hypokinetic areas of myocardium (Figure 2B).¹⁸ Probe accumulation was quantified through the construction of T2* (transverse relaxation) maps *in vivo* (Figure 2C,D) and with fluorescence techniques *ex vivo*.¹⁸ More recently, annexin was conjugated to the surface of a small gadolinium-containing liposome and used to successfully image apoptosis in an isolated perfused rat heart.¹⁹

Myocardial macrophage infiltration is prominent in infarct healing, myocarditis, and transplant rejection. Carbohydrate-coated MNPs are often efficiently phagocytosed by local macrophages and, to a lesser extent and at significantly higher doses, by other cells such as neutrophils.^{20,21} Myocardial macrophage infiltration (Figure 2E-G) has been imaged with MRI, *in vivo*, in animal models of cardiac transplant rejection and in the setting of postinfarction myocardial inflammation.^{22,23} A linear response in the MRI signal was seen in infarcted mice given a wide range of doses (3–20 mg Fe/kg) of the MNPs, consistent with the excellent soft tissue contrast obtainable with MRI.²² A novel myeloperoxidase-activated gadolinium-serotonin chelate has also been developed and shown to detect myeloperoxidase activity in a mouse model of myositis (Figure 2H).²⁴ The ability of this agent to image myeloperoxidase activity in the myocardium is currently being studied.

FLUORESCENCE IMAGING

The imaging armamentarium is being strengthened by the development of fluorescent imaging approaches that will be among the most powerful adjuncts to current techniques. Fluorescence imaging in the myocardium has traditionally been limited to *in vitro* applications, but the development of near-infrared sensors and imaging probes has broadened this technique's scope (Figure 3).^{20,22,25} The main advantages of fluorescence imaging are the stability (i.e. no decay) of the imaging agents, the high (greater than picomolar) sensitivity of the technique, the ability to activate imaging agents and switches *in vivo*, and the capacity to image signals at multiple wavelengths simultaneously (Figure 3D–F).²⁵ The implementation of fluorescence techniques for myocardial imaging in humans, however, will probably require catheter-based, minimally invasive or surgical approaches.

Fluorescence imaging has the potential to provide a wealth of cellular and molecular information.²⁵ Intravital microscopy of fluorescently labeled annexin-V in a mouse model of ischemia-reperfusion powerfully demonstrated the ability of annexin-V to image cardiomyocyte apoptosis during myocardial reperfusion (Figure 4A–C).²⁶ Annexin-V binding to cardiomyocytes occurred within minutes, and maximum binding occurred 20–25 min after the onset of reperfusion. Intraoperative fluorescence imaging of apoptosis during ischemia-reperfusion has also been performed in larger animals.²⁷

Fluorescence imaging of MMP activity has been performed using a long-circulating, quenched, near-infrared fluorescent probe.²⁸ This probe is activated through proteolytic cleavage by MMP2 and MMP9 and has been used *ex vivo* to image the time course of MMP activity after murine myocardial infarction.²⁸ Unlike SPECT imaging of MMPs, the activatable nature of the probe allows MMP substrates to be reacted upon repeatedly, thus amplifying the signal and providing a readout of the enzyme's activity. A similar amplification strategy is used by several other fluorescence imaging agents with potential cardiovascular applications (cathepsins B, S, and K, plasmin, urokinase-type plasminogen activator, and thrombin).

Fluorescence tomography has been used to image postinfarction myocardial inflammation both by directly imaging myocardial macrophage infiltration²² and by imaging the activity of inflammatory proteases such as cathepsin.²⁰ The multispectral capability of fluorescence tomography has also allowed both of these processes to be imaged simultaneously.²⁰ Cysteine protease activity was identified through lysine-lysine cleavage of a protease-activatable sensor (Figure 4D–F) and was eightfold increased in infarcted areas of myocardium.²⁰ Differences in the inflammatory response to ischemia-reperfusion and permanent infarction could also be detected noninvasively in mice through use of this approach. Fluorescence tomography in mice is noninvasive and allows fluorescence activity in the entire mouse heart to be imaged quantitatively and tomographically. In humans, however, fluorescence tomography has low spatial resolution and limited depth penetration, making translation to patients more challenging.

TRANSLATION AND OUTLOOK

Molecular imaging has the potential to impact the treatment of myocardial disease in several ways, such as enhancing the basic understanding of myocardial injury and repair, facilitating the development of novel pharmaceuticals, making possible the early detection of disease (e.g. transplant rejection), and facilitating the use of personalized therapeutic regimens. The translation of targeted molecular imaging agents into clinical use will be cautious and thorough, but several nuclear, magnetic resonance, and optical agents have been used safely in humans.

The high sensitivity of radioisotopes allows low doses of a targeting ligand to be used, reducing the potential for adverse bioeffects and the economic costs of the agent. Nuclear imaging of a

targeted ligand in the myocardium is thus likely to be the quickest route to clinical use. Why, then, pursue the more challenging route of developing molecular MRI agents for targeted myocardial imaging? The rationale for this direction lies chiefly in the attributes of MRI (Table 1) that make it uniquely suited to myocardial imaging. In many centers, MRI has also become the clinical modality of choice for imaging myocardial viability, and the integration of molecular MRI with delayed enhancement, perfusion, strain, and spectroscopic MRI of the myocardium is highly compelling.

Several molecular MRI agents have been successfully used in humans. The MNP ferum-oxides (Feridex; Advanced Magnetix, Cambridge, MA) has been clinically approved for liver imaging since 1993. The long-circulating MNP Combidex (Advanced Magnetix) has been used in humans to image lymph node metastases (phase III trials are completed)²⁹ and, to a lesser extent, inflammation in carotid atherosclerosis.^{30,31} A gadolinium chelate with high affinity for albumin has been approved for clinical use in Europe,³² and a gadolinium chelate labeled with a fibrin-detecting peptide has recently been used to detect thrombus,³³ including intracardiac thrombus, in several patients.³⁴

The potential for clinical translation of fluorescence imaging in the invasive setting is also high. The near-infrared fluorochrome indocyanine green has been used extensively in humans, and it is thus likely that analogous cyanine fluorochromes, currently used for targeted imaging in small animals, will be safe to use in humans, too. Noninvasive fluorescence imaging of the myocardium through the closed chest wall in humans will be challenging, but intraoperative and catheter-based fluorescence systems will allow targeted fluorochromes in the myocardium to be imaged in humans.

Over the next several years, it is likely that we will see ongoing efforts to bring targeted nuclear, MRI and fluorescence imaging agents into clinical use. Dual-modality strategies, such as magnetic resonance with PET or fluorescence,^{35,36} will also be actively pursued. Combination magnetic resonance PET scanners are now being produced for human imaging and will allow MRI and PET imaging to be performed simultaneously, rather than sequentially as is done with PET and CT. The role of targeted molecular imaging in the myocardium is thus likely to continue to grow in both research and clinical settings.

KEY POINTS

- Targeted molecular imaging in the myocardium requires the imaging agent to be small (<50 nm), sensitive (nanomolar to picomolar levels), and able to gain access to the interstitial space
- MRI has been successfully used to image necrosis, apoptosis, macrophage infiltration, and myeloperoxidase activity in the myocardium
- SPECT imaging has been used to visualize necrosis, apoptosis, angiogenesis, and matrix metalloproteinases in injured myocardium
- Fluorescence techniques possess many desirable attributes for molecular imaging of the myocardium but, at present, are limited to invasive approaches in humans
- Several novel imaging agents, suited to targeted molecular imaging in the myocardium, have already completed phase III clinical trials

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Figure 1.

SPECT imaging of molecular targets in the myocardium. (A) Perfusion defect (arrow) in a patient 6–8 weeks after an acute coronary syndrome. (B) Uptake of $^{99\text{m}}\text{Tc}$ annexin-V (arrow) at the time of the event correlates well with the perfusion defect. (C) SPECT imaging of transglutaminase factor XIII activity in a healing murine myocardial infarct. (d) Micro-CT and dual-isotope SPECT imaging of MMP activity in a sham-operated mouse, and (e) in a mouse 1 week after myocardial infarction. Thallium is shown in green, and technetium is shown in red. Absence of thallium uptake indicates injured myocardium; the $^{99\text{m}}\text{Tc}$ probe targeted to MMP has accumulated in these areas. (F) Short-axis image from a dual-isotope SPECT approach with a ^{111}In probe targeted to the $\alpha_5\beta_3$ integrin (red) to detect myocardial angiogenesis (arrows) in dogs 3 weeks after infarction. Absence of $^{99\text{m}}\text{Tc}$ sestamibi (green) delineates perfusion defects. (G) Horizontal, long-axis image of (F). Uptake of the targeted probe (arrow) is highest in the injured hypoperfused areas of myocardium. Permissions obtained: parts A–B, from Elsevier © Hofstra L *et al.* (2000) *Lancet* **356**: 209–212; parts D–E from Lippincott, Williams & Wilkins © Su H *et al.* (2005) *Circulation* **112**: 3157–3167; parts F–G, from American Society for Clinical Investigation © Meoli DF *et al.* (2004) *J Clin Invest* **113**: 1684–1691. Abbreviations: ^{111}In , indium-111-labeled; L, liver; MMP, matrix metalloproteinase; SPECT, single-photon emission CT; $^{99\text{m}}\text{Tc}$, technetium-99m-labeled.

Figure 2.

Targeted molecular MRI in the myocardium. (A) Imaging of cardiomyocyte necrosis (arrow) in an infarcted rat heart, *ex vivo*, with iron-oxide-labeled antimyosin antibodies. (B–D) Molecular MRI of cardiomyocyte apoptosis during ischemia-reperfusion in the mouse heart *in vivo*. (B) T2*-weighted image in a mouse injected with the annexin-labeled apoptosis targeting probe AnxCLIO-Cy5.5 shows accumulation of the probe (arrow) in the hypokinetic anterior wall. (C,D) T2* maps in hypokinetic zones of myocardium show significantly greater probe accumulation (lower T2*) in a mouse injected with (C) AnxCLIO-Cy5.5 than in a mouse injected with (D) CLIO-Cy5.5. (E–G) Molecular MRI of postinfarction myocardial macrophage infiltration in a healing murine infarct. Macrophages in the infarct zone accumulate the macrophage-targeted probe CLIO-Cy5.5, producing (E) marked negative magnetic resonance contrast in the anterior, lateral, and inferolateral walls of the left ventricle. (F) Fluorescence microscopy of probe accumulation correlates well with (G) immunohistochemical staining for macrophages (arrow). (H) Activation of a myeloperoxidase-sensing gadolinium–serotonin chelate in a mouse model of myositis leads to strong positive contrast (arrow) in the area of inflammation. Permissions obtained: part A, from the Radiological Society of North America © Weissleder R *et al.* (1992) *Radiology* **182**: 381–385; parts B–D from John Wiley & Sons © Sosnovik *et al.* (2005) *Magn Reson Med* **54**: 718–724; parts E–G, from Lippincott, Williams & Wilkins © Sosnovik D *et al.* (2007) *Circulation* **115**: 1384–1391; part H, from the Radiological Society of North America © Chen J *et al.* (2006) *Radiology* **240**: 473–481.

Figure 3.

Noninvasive MRI and fluorescence tomography of the mouse heart. (A–C) The fluorescence signal from a depth-resolved slice has been overlaid on a white light image of the mouse. (D–F) Maximum intensity projection images demonstrate the simultaneous imaging of two distinct fluorochromes (biological signals) in an infarcted mouse heart. (A) MRI used for co-localization. (B) Fluorescence signal from a slice intersecting the myocardium in an infarcted mouse injected with the macrophage-targeting probe CLIO-Cy5.5. (C) Sham-operated (noninfarcted) mouse injected with CLIO-Cy5.5. (D) Cathepsin-activated fluorochrome, wavelength 680 nm. (E) Long-circulating intravascular fluorochrome, wavelength 750 nm. (F) Merged signals. Permission obtained for parts A–C from Lippincott, Williams & Wilkins © Sosnovik D *et al.* (2007) *Circulation* **155**: 1384–1391

Figure 4.

Invasive and noninvasive fluorescence imaging approaches. (A–C) Intravital microscopy of cardiomyocyte apoptosis in a mouse with fluorescently labeled annexin-V (green). Microscopic resolution reveals the binding of annexin to the apoptotic cell membrane. Propidium iodide (red) staining of the nucleus confirms the presence of significant cardiomyocyte injury. Similar catheter-based, minimally invasive and open-chest surgical approaches are all translatable to humans. (D) Fluorescence tomography of a cathepsin-activated fluorochrome in an infarcted mouse, indicating the presence of myocardial inflammation. (E) *Ex vivo* fluorescence reflectance imaging of cathepsin activity in the heart correlates well with (F) Triphenyltetrazolium chloride staining of the heart, where the infarcted areas appear pale yellow. Permissions obtained: parts A–C, from Nature Publishing Group © Dumont EA *et al.* (2001) *Nat Med* **7**: 1352–1355; parts D–F, from Lippincott, Williams & Wilkins © Nahrendorf M *et al.* (2007) *Circ Res* **100**: 1218–1225.

Table 1

Attributes of various imaging modalities for anatomical, physiological, and targeted molecular imaging in the myocardium.

Application	Imaging modality		
Molecular imaging	Nuclear	MRI	Fluorescence
Sensitivity	pM	nM	pM
Spatial resolution	+	++++	^a
Activatable probes	–	+++	++++
Multispectral	++	+	++++
Anatomy or physiology	Nuclear	MRI	MDCT
Ejection fraction	+++	++++	+++
Myocardial strain	–	++++	–
Myocardial perfusion	++++	+++	–
Myocardial viability	++++	++++	+
Metabolism	++++	++++	–
Hemodynamics	+	++++	–

^a Refers to noninvasive systems. Invasive systems (e.g. fiberoptic microscopes) can achieve microscopic resolution.

Abbreviations: –, not capable; +, limited capability; ++, good performance in selected circumstances; +++, high performance; +++++, excellent performance/gold standard; MDCT, multidetector X-ray CT.