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Molecular and Microstructural Imaging of the Myocardium

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Abstract

The past year has witnessed ongoing progress in the field of molecular MRI of the myocardium. In addition, several novel fluorescent agents have been introduced and used to image remodeling in the injured myocardium. New techniques to image myocardial microstructure, such as diffusion spectrum MRI, have also been introduced and have tremendous potential for integration and synergy with molecular MRI. In the current review we focus on these and other advances in the field that have occurred over the past year.

Keywords

Myocardium; MRI; Molecular imaging; Diffusion; Microstructure

Introduction

The ability of MRI to evaluate myocardial function, perfusion, and viability has made it a powerful tool in numerous clinical and research applications. Moreover, over the past year the scope and role of MRI of the myocardium has continued to grow. The understanding of techniques exploiting endogenous contrast in the myocardium, such as arterial spin labeling [1], blood oxygen level-dependent imaging [2], and magnetization transfer [3], has improved significantly, and these techniques are moving steadily toward clinical translation. Moreover, significant advances have been made in molecular MRI of the myocardium, including new techniques to image ischemia, cell death, left ventricular remodeling, heart failure, and transplant rejection.

Advances in cellular and molecular imaging of the myocardium over the past year have been accompanied by significant advances in microstructural imaging of the myocardium with diffusion-encoded MRI. Microstructural imaging of the myocardium images the heart at the scale of individual myofiber tracts and sheets and thus has the potential to provide a mechanistic bridge between events at the cellular/molecular level and global (whole organ) physiology. This technique thus adds further scope to the armamentarium of cardiac MRI and its ability to obtain comprehensive and integrated datasets of the myocardium in health and disease. In the current article we provide an update on key advances in the field over the past 12 months. We focus primarily on new magnetic resonance—and fluorescence-based techniques for molecular and micro-structural imaging of the myocardium. The reader interested in more background on the field is referred to our recent review in this journal [4],

as well as other recent publications in the field [5,6,7•]. Advances in molecular imaging of the myocardium using radiolabeled probes are covered elsewhere in this issue.

Microstructural Imaging of the Myocardium

The microstructure of the myocardium greatly affects cardiac mechanics. In particular, the orientation and helix angle of myofiber tracts in the myocardium play a central role in the generation of ventricular torsion and myocardial strain. In a series of landmark articles, Streeter and colleagues [8,9] showed histologically that the myocardium consisted of an array of crossing helical myofibers. MRI has the unique ability to image nerve and muscle tracts noninvasively by measuring the characteristic diffusion of water in biological tissue. Initial implementations of diffusion MRI involved the use of magnetic resonance diffusion tensor imaging (DTI) to approximate the three-dimensional fiber structure in the heart noninvasively [10–13]. DTI has been successfully used to investigate infarct healing and myocardial remodeling after ischemic heart injury in various animal species [14,15,16••,17]. Micro-structural changes were found in terms of mean diffusivity, fractional anisotropy, and fiber orientation in the infarct region. It should be noted that these studies were conducted mainly in excised hearts. However, in vivo imaging of myocardial microstructure using DTI is highly feasible and an area of active technology development.

In a landmark clinical paper, Wu et al. [18] used DTI in conjunction with delayed-enhancement MRI to study myocardial microstructure in patients with recent/acute myocardial infarction. In a subsequent article, the authors recently reported the use of DTI to image sequential changes in myocardial microstructure during the period of infarct healing and remodeling [19••]. A decrease in mean diffusivity in recent versus healed infarcts was documented, and was thought to reflect the recovery of tissue integrity. The remodeling of fiber architecture in the remote zone, as reflected by a more right-handed helical orientation, was associated with improved wall thickening. This study is the first of its kind to serially investigate infarct healing and myocardial remodeling after ischemic heart injury in a clinical setting using the DTI technique [19••]. Such studies have the potential to greatly improve our knowledge of ventricular microstructure and the remodeling of myofiber architecture in response to infarction.

A further significant advance in microstructural imaging of the myocardium was reported in the past year and involved the application of diffusion spectrum MRI tractography (DSI tractography) in the heart [20••]. DSI is the most robust (assumption free, highly generalizable) of a group of techniques that sample diffusion or q-space with high angular resolution [21•]. Unlike DTI that derives a primary diffusion vector (primary eigenvector) in each imaging voxel, DSI tractography involves the integration of multiple diffusion vectors per voxel to yield detailed and continuous fiber tracts. The reader interested in a more detailed description of the basis of DSI and DTI, and the merits of the two techniques, is referred to recent articles in the field [21•,22]. In the context of this article it suffices to say that the attributes of DSI provide a platform to image myocardial fiber architecture with an unheralded level of detail and accuracy. In a recent study involving normal and infarcted rat hearts [20••], DSI tractography was able to robustly resolve both normal myofiber anatomy as well as complex and converging myofiber patterns in the infarct zone (Fig. 1).

In normal myocardium, myofibers form a series of crossing helical structures, in which the helix angle (angle at which the myofiber spirals around the long axis of the left ventricle) varies smoothly from a left-handed helix (0° to negative 90°) in the subepicardium to a right-handed helix (0° to 90°) in the subendocardium [8,9]. Using DSI tractography, the detailed three-dimensional architecture of normal myocardium could be imaged nondestructively in an intact heart for the first time [20••]. Myofibers in the mid-

myocardium were aligned circumferentially around the long axis of the left ventricle (zero helix angle), subendocardial fibers had a positive or right-handed helix angle, and myofibers in the subepicardium had a negative or left-handed helix angle. The transition in fiber helix angle from endocardium to epicardium occurred in a smooth and symmetrical manner, with little dispersion in the helix angle at a given transmural plane [20••].

Myofiber architecture in the infarcted hearts was severely disrupted (Fig. 1). However, numerous residual myofibers were present within the infarcts and, in the more basal and septal portions of the infarct, often formed a mesh-like network of orthogonal myofibers [20••]. Orthogonal myofibers within these residual myofiber networks frequently lay in direct contact with each other, forming nodes of myofiber contact [20••]. The implications of such myofiber patterns in infarcted myocardium remain to be determined. It is likely, however, that residual myofiber networks resist mechanical remodeling while at the same time increasing the risk for lethal reentrant arrhythmias.

The application of diffusion-encoded MRI in the myocardium to date has been principally *ex vivo*. However, DTI of the myocardium has been performed in normal volunteers and patients *in vivo* [18,19••]. Recent technical advances in radiofrequency excitation and reception make it fairly likely that DSI tractography of the heart will soon become feasible *in vivo* as well [23••,24]. Together with advances in molecular imaging, the application of these microstructural imaging techniques will advance our understanding of the mechanical and electrical properties of the myocardium and help delineate the molecular and microstructural basis of myocardial injury.

Molecular Imaging of the Myocardium

The past year has witnessed significant growth in techniques to perform molecular imaging of the myocardium. Several new target-specific agents have been developed and applied to study a range of cardiac diseases in both small and large animal models. The use of these molecular imaging probes has improved the understanding of pathological changes and molecular processes in the myocardium. Moreover, the sensitivity and target specificity shown by these agents in preclinical studies have important implications for clinical translation of molecular imaging in the heart and other organs in general.

New Fluorescent and Magnetofluorescent Imaging Agents

The use of several new fluorescent imaging agents in the myocardium has been reported over the past year. The near-infrared fluorochrome Cy5.5 was conjugated to the RGD peptide and used to image integrin expression on myofibroblasts in healing infarcts [25••]. Myofibroblasts play an important role in left ventricular fibrosis, and a radiolabeled version of the Cy5.5-RGD probe was thus developed to follow this *in vivo* [25••]. In addition, the impact of angiotensin-converting enzyme inhibition and/or blockade on left ventricular remodeling could be successfully imaged with the agent [25••]. In another study, a fluorescently labeled angiotensin peptide was used to image angiotensin receptor expression in a mouse model of ischemic cardiomyopathy [26]. Intravital microscopy was used to image the angiotensin receptor expression at various time points after myocardial infarction [26]. These studies have provided valuable insights into the pathophysiology and treatment of myocardial remodeling.

Iron oxide nanoparticles and gadolinium-loaded nano-particles can be easily converted into magnetofluorescent imaging agents without changing their properties or pharmacokinetics. Conjugation of a fluorochrome to small gadolinium chelates, such as gadolinium diethylenetriamine pentaacetic acid (Gd-DTPA), however has been difficult to achieve without drastically changing their pharmacokinetics. Recently, however, the synthesis of a

novel fluorescent small gadolinium chelate, Gd-DTPA-NBD, has been described [27••]. Unlike most organic fluorochromes, NBD is small, with no charge, and has minimal protein binding potential. These properties ensure that the kinetics of Gd-DTPA-NBD remain similar to those of Gd-DTPA and suitable for delayed enhancement imaging of the myocardium [27••]. Gd-DTPA-NBD, like Gd-DTPA, does not cross the intact cell membrane and accumulates in areas of cell rupture and myocardial necrosis. The accumulation of Gd-DTPA-NBD, however, can also be detected fluorescently and with immunohistochemistry, as shown in the infarcted mice in Fig. 2 [27••]. Immunohistochemical staining for NBD showed that the chelate accumulated only in the expanded extracellular space of the infarcted myocardium (Fig. 2). This chelate thus allowed the basis and mechanism of delayed enhancement imaging to be resolved at the cellular level [27••]. More generally, the use of NBD provides an easy and convenient alternative to the use of europium to characterize the cellular uptake of small gadolinium chelates.

New Developments in Molecular Imaging of Ischemic Myocardial Injury

The use of a collagen-binding gadolinium chelate, EP-3600, to image chronic myocardial scar has previously been described [28]. It was recently reported that this agent was used to visualize myocardial perfusion defects in a large animal model with non-occlusive coronary artery stenosis [29••]. The binding of the agent to collagen in the extracellular matrix of the myocardium is a function of perfusion/delivery, and the use of EP-3600 facilitated prolonged visualization of perfusion defects (up to 60 min after contrast agent administration) [29••]. The use of EP-3600 thus allowed myocardial perfusion to be imaged under steady-state conditions, overcoming many of the limitations associated with first-pass perfusion imaging.

Apoptosis plays a central role in the loss of functional cardiomyocytes (CMs) during myocardial ischemia and reperfusion. Currently, the detection of apoptotic CMs is based on the binding of annexin-conjugated molecular imaging probes to phosphatidylserine expressed on the outer surface of the apoptotic cell membrane. However, in regions where there is co-existent myocardial necrosis, the uptake of annexin-conjugated probes due to CM apoptosis cannot be distinguished from its uptake due to CM necrosis, making the technique a marker of composite cell death rather than a specific assay of CM apoptosis. A dual-contrast molecular MRI technique to distinguish apoptotic and necrotic myocytes, however, has recently been reported [27••]. The technique involves the use of the annexin-based nanoparticle AnxCLIO-Cy5.5 to image CM apoptosis and Gd-DTPA-NBD to image CM necrosis (Fig. 3) [27••].

In addition to imaging both CM apoptosis and necrosis simultaneously, this newly described technique allowed CM apoptosis to be imaged within the first 4 to 6 h of ischemic injury [27••]. This is a significantly earlier time point than used in previous studies and is the period during which most cell death occurs. CM apoptosis in mice exposed to transient coronary artery ligation developed most frequently in the mid-myocardium, while CM necrosis appeared earliest in the subendocardium [27••]. In addition, most apoptotic CMs within 4 to 6 h of ischemia-reperfusion remained potentially viable, and provided an important target for myocardial salvage [27••]. This dual contrast agent molecular MRI technique is based on the different tissue relaxation effects of the two contrast agents. Because these relaxation effects are field dependent, different imaging parameters will be needed to balance the $R2/R2^*$ and $R1$ effects at different field strengths. Although promising, further study will be needed to determine the utility of the dual-contrast approach at lower field strengths and in the clinical setting.

Molecular Imaging in Heart Failure and Transplantation

CM apoptosis plays an important role in the development and progression of heart failure. However, the level of apoptosis in heart failure is substantially lower than that seen in acute conditions such as ischemia and transplant rejection. This makes in vivo molecular imaging of apoptosis in heart failure highly challenging, particularly with molecular MRI because of its lower sensitivity in comparison with nuclear imaging techniques. Recently, however, the feasibility and sensitivity of detecting low levels of CM apoptosis with molecular MRI was demonstrated in a transgenic model of chronic heart failure using the apoptosis-sensing nanoparticle, AnxCLIO-Cy5.5 [30••]. This study showed that low levels (1%–2%) of CM apoptosis could be imaged in vivo in postpartum Gaq-overexpressing mice (Fig. 4) [30••]. These mice develop a postpartum cardiomyopathy, which closely recapitulates a variant of heart failure seen in humans. The in vivo uptake of AnxCLIO-Cy5.5 in the mice, as measured by the reduction of T2*, correlated strongly with myocardial caspase-3 activity, demonstrating the sensitivity and specificity of the agent for CM apoptosis [30••]. The results of this study demonstrate the ability of iron oxide nanoparticles less than 50 nm in size to cross normal/nonischemic capillary membranes and image sparsely expressed molecular targets in the myocardium in heart failure.

The detection of transplant rejection remains a significant problem and currently requires serial invasive endomyocardial biopsies. In transplant rejection, macrophages function as inflammatory amplifiers and constitute a large part of the cellular infiltrate, causing tissue damage and graft rejection. In vivo imaging of transplant rejection has thus previously been performed using magnetic nanoparticles to image macrophage infiltration in vivo [31]. Recently, molecular imaging of transplant rejection was performed in a mouse model of transplant rejection using a fluorescent reporter of protease activity and a macrophage-avid magnetofluorescent nanoparticle [32]. Both fluorescent imaging and molecular MRI were able to detect macrophage accumulation in transplant rejection noninvasively. Moreover, a good correlation was found between the in vivo imaging results and the degree of allograft rejection [32], further demonstrating the potential of molecular imaging to noninvasively identify and quantify myocardial transplant rejection.

Conclusions

The role of molecular and microstructural MRI in preclinical investigation and basic science continues to grow. The past year has witnessed ongoing advances in both arenas, with the introduction of several new techniques and agents. These advances have enhanced the ability of noninvasive imaging to increase our understanding of cardiovascular disease and facilitate the development of novel cardioprotective therapies. The potential of combining molecular and microstructural imaging is particularly appealing and has the potential of linking events at the subcellular level to changes in whole-organ pathophysiology. Clinical translation of microstructural MRI techniques is highly feasible and is being actively pursued. Likewise, several new molecular MRI agents have been approved in the past year. These include an albumin-binding gadolinium chelate (Vasovist; Epix Pharmaceuticals, Cambridge, MA) and a long circulating iron oxide nanoparticle (Ferumoxytol; Advanced Magnetix, Cambridge, MA). The translation of molecular MRI agents, while challenging, is thus certainly feasible and will add tremendous value to the already expanding armamentarium of cardiovascular MRI.

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Fig. 1. Diffusion spectrum MRI tractography of the myocardium [20••]. The myofiber tracts are color-coded by the helix angle they make with the long axis of the left ventricle. The images in the *upper row* are of a normal rat heart viewed in its short axis and from its lateral wall. The transmural evolution in myofiber helix angle is clearly seen. In normal myocardium, myofibers with orthogonal helix angles course in separate transmural planes and do not make contact. Two infarcted rat hearts are shown in the *bottom row*. Fiber architecture in the infarct is severely perturbed, but numerous residual myofibers are present. Moreover, the residual myofibers in the infarct frequently have orthogonal helix angles and can form nodes of fiber contact or intersection. (From Sosnovik et al. [20••]; with permission)

Fig. 2. Delayed enhancement imaging of an infarcted mouse heart with GD-DTPA-NBD [27••]. Volume-rendered three-dimensional (3D) MRI of the bisected heart clearly shows that the agent has accumulated in the infarct zone. This is confirmed with a planar reformat of the MRI dataset and with fluorescence reflectance imaging (FRI). Immunohistochemistry for the NBD moiety on the agent is shown in the *right* column at both low and high magnification. The agent accumulates in the expanded extracellular space of necrotic myocardium. GD-DTPA—gadolinium diethylenetriamine pentaacetic acid. (From Sosnovik et al. [27••]; with permission)

Fig. 3. Simultaneous imaging of cardiomyocyte apoptosis and necrosis with molecular MRI [27••]. Areas of myocardium with apoptotic and/or necrotic cardiomyocytes accumulate AnxCLIO-Cy5.5 (*left column*). However, only areas of necrosis also show delayed enhancement with Gd-DTPA-NBD (*red arrows, middle column*). Immunohistochemistry for NBD confirms that Gd-DTPA-NBD accumulates only in necrotic myocardium, where cell rupture has resulted in the expansion of the extracellular space (*right column*). GD-DTPA—gadolinium diethylenetriamine pentaacetic acid; LV—left ventricular. (From Sosnovik et al. [27••]; with permission)

Fig. 4.

Molecular MRI of cardiomyocyte apoptosis in heart failure [30••]. Postpartum Gaq-overexpressing mice with heart failure have been used. These mice have very low levels of apoptosis (1%–2%), minimal inflammation and necrosis, and normal capillary membrane permeability. Nevertheless, cardiomyocyte apoptosis can be imaged in vivo in this model with AnxCLIO-Cy5.5, as shown in the T2* maps and T2*-weighted images. T2* is significantly reduced in the animals injected with AnxCLIO-Cy5.5, and numerous discrete foci of probe uptake (signal hypointensity, *white arrows*) are seen. Fluorescence microscopy of a mouse injected with AnxCLIO-Cy5.5 confirms the uptake of the agent by apoptotic cardiomyocytes (*yellow arrows*). (From Sosnovik et al. [30••]; with permission)