Molecular MRI of Cardiomyocyte Apoptosis With Simultaneous Delayed-Enhancement MRI Distinguishes Apoptotic and Necrotic Myocytes In Vivo
Potential for Midmyocardial Salvage in Acute Ischemia

Background—A novel dual-contrast molecular MRI technique to image both cardiomyocyte apoptosis and necrosis in vivo within 4 to 6 hours of ischemia is presented. The technique uses the annexin-based nanoparticle AnxCLIO-Cy5.5 (apoptosis) and simultaneous delayed-enhancement imaging with a novel gadolinium chelate, Gd-DTPA-NBD (necrosis).

Methods and Results—Mice with transient coronary ligation were injected intravenously at the onset of reperfusion with AnxCLIO-Cy5.5 (n=7) or the control probe Inact_CLIO-Cy5.5 (n=6). T2*-weighted MR images (9.4 T) were acquired within 4 to 6 hours of reperfusion. The contrast-to-noise ratio between injured and uninjured myocardium was measured. The mice were then injected with Gd-DTPA-NBD, and delayed-enhancement imaging was performed within 10 to 30 minutes. Uptake of AnxCLIO-Cy5.5 was most prominent in the midmyocardium and was significantly greater than that of Inact_CLIO-Cy5.5 (contrast-to-noise ratio, 8.82±1.5 versus 3.78±1.1; P<0.05). Only 21±3% of the myocardium with accumulation of AnxCLIO-Cy5.5 showed delayed-enhancement of Gd-DTPA-NBD. Wall thickening was significantly reduced in segments with delayed enhancement and/or transmural accumulation of AnxCLIO-Cy5.5 (P<0.001). Fluorescence microscopy of AnxCLIO-Cy5.5 and immunohistochemistry of Gd-DTPA-NBD confirmed the presence of large numbers of apoptotic but potentially viable cardiomyocytes (AnxCLIO-Cy5.5 positive, Gd-DTPA-NBD negative) in the midmyocardium.

Conclusions—A novel technique to image cardiomyocyte apoptosis and necrosis in vivo within 4 to 6 hours of injury is presented and reveals large areas of apoptotic but viable myocardium in the midmyocardium. Strategies to salvage the numerous apoptotic but potentially viable cardiomyocytes in the midmyocardium in acute ischemia should be investigated.

Key Words: molecular imaging ■ MRI ■ apoptosis ■ myocardium ■ ischemia

A poptosis plays a central role in the loss of functional cardiomyocytes (CMs) during myocardial ischemia and reperfusion.1,2 In a pioneering study, intravitral microscopy of fluorescently labeled annexin-V was used to image CM apoptosis in mice within 30 minutes of ischemia-reperfusion.3 In an equally impressive clinical study,99mTc-annexin was used to image CM death in mice within 30 minutes of ischemia-reperfusion.4 The spatial resolution of SPECT, however, is poor, and the dynamic range of the technique was limited in the 12 hours after probe injection by high background signal.4 However, it is precisely during the first 4 to 6 hours of ischemic injury that CM apoptosis is most prevalent and important to image.3,5 The uptake of 99mTc-annexin due to CM apoptosis could also not 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.4,6

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Molecular MRI of CM apoptosis has the potential to overcome some of these limitations. We have previously reported the synthesis of an annexin-labeled magnetofluorescent nanoparticle, AnxCLIO-Cy5.5, and have shown that the agent can be used for high-resolution imaging of CM apoptosis/death in vivo 24 hours after injury.7 In the present study, we describe the use of AnxCLIO-Cy5.5 to image CM apoptosis within the first 4 to 6 hours of ischemia, the period...
during which apoptosis is most prevalent.\textsuperscript{5} In addition, a novel dual-contrast MRI approach using AnxCLIO-Cy5.5 and delayed-enhancement (DE) imaging of a novel magnetofluorescent gadolinium chelate is introduced. This dual-contrast technique is shown to be capable of distinguishing CM apoptosis from CM necrosis in vivo and thus capable of identifying myocardium that is potentially amenable to salvage in acute ischemia.

Mice exposed to transient coronary artery ligation were imaged in this study with the developed dual-contrast MRI approach. CM apoptosis after ischemia-reperfusion was most frequently seen in the subendocardium, whereas CM necrosis was usually confined to the subendocardium. The results of the study show that the described dual-contrast approach is robust and that the majority of annexin-positive CMs seen within 4 to 6 hours of ischemia are apoptotic, viable, and may be potentially salvageable. The presented dual-contrast MR approach overcomes several major limitations previously associated with apoptosis imaging in vivo including the ability to image CM apoptosis within the first 4 to 6 hours of injury with high specificity and spatial resolution. The approach described is highly translatable, has the potential to provide novel insights into mechanisms of CM death and survival, and is thus of both broad scientific and clinical relevance.

Methods

Imaging Agents and Techniques

The basis of the dual-contrast approach used in the study lies in the accumulation of gadolinium-DTPA in areas of necrotic myocardium,\textsuperscript{8} where rupture of the necrotic cell membranes increases the extracellular volume fraction available to the probe.\textsuperscript{8} In contrast, potentially salvageable CMs during the early phase of apoptosis have intact cell membranes and thus do not accumulate cell-impermeable agents such as Gd-DTPA.\textsuperscript{8,9} The feasibility of the dual-contrast approach is based on several technical innovations, including (1) the use of an ultrahigh performing 1500 mT/m gradient system allowing echo times (TE) < 1 ms to be achieved in mice at 9.4 T, (2) use of a low dose of AnxCLIO-Cy5.5 (4 mg Fe/kg) to prevent extremely high local concentrations of iron oxide, (3) synthesis of active and inactive imaging agents with blood half-lives of 2 to 3 hours, and (4) the use of a novel fluorescent gadolinium chelate.

The synthesis and properties of AnxCLIO-Cy5.5 have been previously described.\textsuperscript{10} It should be noted, however, that the transverse relaxivity (R2) of the current agent is >80 mmol/L·1 s\textsuperscript{-1}. AnxCLIO-Cy5.5 was injected at the onset of reperfusion and the gadolinium chelate within 4 to 5 hours of reperfusion. A novel fluorescently labeled small gadolinium chelate, gadolinium-DTPA-NBD (Gd-DTPA-NBD), was synthesized by attaching DTPA and NBD to a dipeptide scaffold and allowed the presence of DE to be confirmed histologically.\textsuperscript{11} The transverse/longitudinal relaxivity (R2/R1) ratio of AnxCLIO-Cy5.5 approaches 80 at 9.4 T.\textsuperscript{12} DE imaging was thus performed with a TE of 1 ms to neutralize the R1 and R2 effects of AnxCLIO-Cy5.5 and produce an image suitable for the detection of the T1 effects of Gd-DTPA-NBD.

Fluorescent labeling of small gadolinium chelates has been difficult to achieve without drastically altering their pharmacokinetics. Most organic fluorochromes are significantly larger than Gd-DTPA, and are highly charged, and have the potential to bind to plasma proteins. NBD, however, is significantly smaller than most organic fluorochromes, has no charge, and has minimal potential for protein binding. Conjugation of NBD to the small molecule wortmannin did not significantly alter its kinetics or biological activity.\textsuperscript{11} The Gd-DTPA-NBD construct was thus chosen to maintain the properties of Gd-DTPA and the pharmacokinetic basis of DE imaging.

Experimental Protocol

Eighteen wild-type C57BL/6 mice were studied. Three mice were used to determine the blood half-lives of AnxCLIO-Cy5.5 and the control probe, Inact_CLIO-Cy5.5, on which the annexin moiety had been inactivated. The conversion of AnxCLIO-Cy5.5 to Inact_CLIO-Cy5.5 was produced by exposure to acetic anhydride, and loss of annexin activity was confirmed by flow cytometry of apoptotic Jurkat T cells. The active and control probes thus had identical sizes, relaxivities, and physical properties. The blood half-lives of the agents were determined, using a mono-exponential decay model, from fluorescence measurements of serial blood draws.

Permanently myocardial infarctions were produced in 2 of the mice to test the pharmacokinetics of DE imaging with Gd-DTPA-NBD. Seventy-two hours after infarction, the mice were injected intravenously with 0.3 mmol/kg of Gd-DTPA-NBD and euthanized 20 minutes later. The excised hearts were bisected in the short axis of the left ventricle, embedded in OCT, and sectioned for immunohistochemical detection of Gd-DTPA-NBD. Ten 5-μm-thick cryosections were acquired from each tissue block. The remainder of the tissue block was then thawed for fluorescence reflectance imaging (FRI) and MR microscopy. FRI was performed with a 12-bit CCD camera (Kodak, Rochester, NY) and FITC filter, which was used for the detection of NBD. The tissue blocks were then placed together in a fluorocarbon MR matching medium and imaged with a T1-weighted 3D gradient echo sequence at 9.4 T. Imaging parameters were as follows: field of view, 12.8 mm; matrix, 196; spatial resolution, 65 μm isotropic; repetition time, 20 ms; TE, 2.9 ms; flip angle, 52°; number of excitations (Nex), 4; and acquisition time, 51 minutes. Postprocessing of the FRI and MRI datasets was performed using Osirix imaging software (Freesw, University of Geneva).

The remaining 13 mice were exposed to transient coronary ligation (35 minutes) followed by reperfusion. The mice were injected intravenously with 4 mg Fe/kg of either AnxCLIO-Cy5.5 (n=7) or the control probe Inact_CLIO-Cy5.5 (n=6) at the onset of reperfusion. In half the mice in each group, the location of the coronary ligation was chosen to produce ischemia in 30% to 40% of the left ventricle (mild–moderate injury) and in the other half to produce ischemia in 60% to 75% of the ventricle (severe–extensive injury). This strategy allowed the sensitivity and specificity of AnxCLIO-Cy5.5 to be assessed over a wide range of injury.

In vivo MR images were acquired within 4 to 6 hours of reperfusion on a 9.4 T horizontal bore magnet (Biospec, Bruker, Billerica Mass). Cardiac gated (SA Instruments, Stonybrook, NY) gradient echo cines were acquired in the short axis of the left ventricle from the point of coronary ligation to the apex using echo times of 1.25, 1.5, and 1.75 ms. Other parameters were as follows: field of view, 25×25 mm; slice, 1 mm; matrix, 200×200 (125-μm resolution); flip angle, 30°; and Nex, 4. After completion of the T2*-weighted acquisitions to detect AnxCLIO-Cy5.5, the mice were injected intravenously with 0.3 mmol/kg of Gd-DTPA-NBD. T1-weighted DE imaging was performed 10 to 30 minutes after injection using the identical parameters, but with a flip angle of 60°. The mice were then immediately euthanized, and the hearts embedded for fluorescence microscopy, histology, and immunohistochemistry.

CM apoptosis was identified histologically with a terminal uridine nick-end labeling (TUNEL) assay (Integreen, New York, NY). In the mice injected with AnxCLIO-Cy5.5, cell counting was performed (>160 high-power fields) to determine the percentage of TUNEL-positive cells that were CMs versus interstitial cells. Fluorescence microscopy of AnxCLIO-Cy5.5 uptake was performed using the following filters: excitation, 650±22.5 nm; emission, 680 nm longpass and 710±25 nm bandpass. Immunohistochemical detection of Gd-DTPA-NBD was performed with a primary polyclonal rabbit anti 4-fluoro-7-nitrobenzofurazan antibody (AbD Serotec, Raleigh, NC).\textsuperscript{14} After washing with PBS, secondary biotinylated anti-rabbit IgH (H+L) (Vector Laboratories, Burlingame, Calif) antibody was applied, followed by avidin-peroxidase complex (Vectorstain ABC kit, Vector Laboratories). The reaction was visualized with 3-amino-9-ethyl carbazole substrate (AEC; Sigma, St Louis, Mo), and the

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sections were counterstained with Mayer hematoxylin solution (Sigma).

The uptake of AnxCLIO-Cy5.5 and Inact_CLIO-Cy5.5 were compared (unpaired t test and Mann–Whitney, Prism, Graphpad, La Jolla, Calif) by measuring the contrast-to-noise ratio (CNR) between the injured myocardium and the uninjured septum. We have previously shown that this value responds linearly to probe concentrations and correlates extremely well with fluorescence measurements of probe uptake.14 The region of injured myocardium was defined by the presence of regional wall motion abnormalities on cine MRI. Percent wall thickening (systolic wall thickness/diastolic wall thickness)/diastolic wall thickness was measured in those segments with probe uptake. An unpaired t test was used to compare percent wall thickening (PWT) in segments with and without DE. The transmural extent of AnxCLIO-Cy5.5 accumulation was divided into 3 groups (<33%, 33% to 66%, and ≥66%). PWT in these groups was compared using an ANOVA with a Tukey post test comparison. All studies were performed in accordance with the guidelines for the humane care of research animals at our institution.

Results

The blood half-life of AnxCLIO-Cy5.5 averaged (mean±SEM) 2.7±0.4 hours, whereas that of Inact_CLIO-Cy5.5 averaged 2.9±0.6 hours (P=0.76, unpaired t test). In all mice injected with AnxCLIO-Cy5.5, strong accumulation of the agent was seen in the hypokinetic and akinetic areas of myocardium. No accumulation of AnxCLIO-Cy5.5 was seen in segments of myocardium with normal contraction. In those mice with milder injury (more apical coronary ligation), accumulation of AnxCLIO-Cy5.5 was best visualized at a TE of 5.5 ms. However, in the mice with more severe injury (more basal coronary ligation), a TE of 2.5 to 4 ms permitted robust visualization of the probe (Figure 1). The R2 and R1 effects of AnxCLIO-Cy5.5 were balanced at a TE of 1 ms, and neither signal hypointensity nor hyperintensity were seen at this TE, even in those areas with marked accumulation of AnxCLIO-Cy5.5 (Figure 1). DE imaging of Gd-DTPA-NBD could thus be robustly performed in all mice at a TE of 1 ms.

DE imaging of the 2 infarcted mice revealed that the kinetics of Gd-DTPA-NBD were not altered by the NBD moiety. Twenty minutes after injection, complete washout of the agent was seen in areas of normal myocardium, whereas profound DE was seen in infarcted myocardium (Figure 2). The distribution of Gd-DTPA-NBD by MRI correlated strongly with FRI and immunohistochemistry of the agent (Figure 2), which revealed that Gd-DTPA-NBD accumulated in areas of CM necrosis where the extracellular volume fraction was increased (Figure 2). No accumulation of Gd-DTPA-NBD was seen within CMs or in areas where the CM cell membrane was intact. The accumulation of AnxCLIO-Cy5.5 was most frequent and prominent in the midmyocardium (Figure 3). In those mice with mild-moderate injury, no significant accumulation

Figure 1. Molecular MRI of CM apoptosis with AnxCLIO-Cy5.5 4 to 6 hours after transient left coronary artery ligation. Detection of the agent is strongly modulated by the TE used. At a TE of 1 ms at 9.4 T (A), the R1 and R2 effects of AnxCLIO-Cy5.5 balance each other, and the injured and uninjured areas of the myocardium are isointense. As the TE is increased to 2.5 ms (B) and 4 ms (C), signal hypointensity caused by the accumulation of AnxCLIO-Cy5.5 in the injured myocardium becomes clearly visible (yellow arrows).

Figure 2. DE MRI of Gd-DTPA-NBD in an infarcted mouse heart. The heart was excised 20 minutes after the injection of Gd-DTPA-NBD, bisected, and imaged with a T1-weighted 3D gradient echo sequence. A volume-rendered image (A) and a 2D short-axis reconstruction at midventricular level (B) are shown. Accumulation of Gd-DTPA-NBD is seen in the territory of the ligated left coronary artery (anterior wall to inferolateral wall and most of the apex). C, FRI of Gd-DTPA-NBD shows that the detection of the agent by MRI and by fluorescence imaging correspond very well. D (magnification ×100) and E (×400), Immunohistochemistry for Gd-DTPA-NBD at the border zone of the infarct shows that the agent accumulates in areas of CM degeneration and expansion of the extracellular space. No accumulation of the agent is seen in areas with intact CM cell membranes.
AnxCLIO-Cy5.5 than Inact_CLIO-Cy5.5 (8.82 ms) was significantly higher in the mice injected with TEs (Figures 3 and 4). CNR from probe accumulation (TE, 4 ms) was dramatically higher than that of Inact_CLIO-Cy5.5 in all mice and at all TEs (Figures 3 and 4). CNR from probe accumulation (TE, 4 ms) was significantly higher in the mice injected with AnxCLIO-Cy5.5 than Inact_CLIO-Cy5.5 (8.82 ± 1.5 versus 3.78 ± 1.1; P < 0.02, unpaired t test; P = 0.03, Mann–Whitney).

Fluorescence microscopy confirmed the in vivo MRI findings. A strong concordance was seen between probe distribution in vivo and by microscopy (Figure 4). Moreover, fluorescence microscopy revealed the presence of numerous morphologically intact CMs decorated with AnxCLIO-Cy5.5 (Figure 4), consistent with the active binding of the agent to apoptotic CMs. In areas of myocardium with mild-moderate injury, the uptake of AnxCLIO-Cy5.5 was predominantly midmyocardial, and minimal uptake of Gd-DTPA-NBD was seen (Figure 5). TUNEL-positive cells were most frequently seen in the midmyocardium, and 83.1 ± 2.7% of TUNEL-positive cells were CMs (Figure 5). The uptake of AnxCLIO-Cy5.5 in the midmyocardium was thus predominantly by apoptotic CMs (Figure 5).

In areas of myocardium with severe injury, DE of Gd-DTPA-NBD was seen, particularly in the subendocardium (Figure 6). The extent of DE was usually mild at the midventricular level, increased progressively in the more apical portions of the myocardium, and could be fairly extensive at the apex (Figure 6). Immunohistochemistry for Gd-DTPA-NBD confirmed the in vivo DE findings (Figure 6). Overall in the 7 mice injected with AnxCLIO-Cy5.5, 21 ± 3% (mean ± SEM) of myocardium with AnxCLIO-Cy5.5 accumulation also showed positive DE of Gd-DTPA-NBD (Figure 7). PWT was significantly better (21.0 ± 2.4% versus 6.3 ± 1.1%, P < 0.0001) in those segments without DE versus those with DE (Figure 7). PWT averaged 35.6 ± 3.3%, 18.9 ± 1.6%, and 7.9 ± 1.3% in segments with AnxCLIO-Cy5.5 involving <33%, 33% to 66%, and >66% of transmural thickness, respectively (P < 0.01 for all post test comparisons). A clear relationship was thus seen between the transmural extent of AnxCLIO-Cy5.5 and myocardial contractility (Figure 7).

**Discussion**

Molecular imaging of CM apoptosis has the potential to facilitate the development of novel cardioprotective strategies but has been hampered to date by low spatial resolution, poor dynamic range during the period of maximal CM apoptosis, and the inability to distinguish CM apoptosis from CM necrosis.4,6,15,16 The dual contrast molecular MRI approach presented in this study overcomes these limitations and has the potential to provide new insights into CM death after ischemia. We show in this study, in a mouse model of ischemia-reperfusion, that CM

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**Figure 3.** Molecular MRI (TE, 4 ms) of CM apoptosis in myocardium exposed to mild-moderate injury. A, Mouse injected with AnxCLIO-Cy5.5. B, Mouse with a similar degree of injury but injected with the control (annexin-inactivated) agent Inact_CLIO-Cy5.5. Significant midmyocardial uptake of the active probe (signal hypointensity) is seen in the region of injury (yellow arrows). No significant uptake of the control probe is seen (B).

**Figure 4.** Molecular MRI of CM apoptosis (TE, 4 ms) within 4 to 6 hours of ischemia-reperfusion in mice with severe and extensive injury. A and B, Mouse injected with AnxCLIO-Cy5.5. C and D, Mouse injected with the control probe Inact_CLIO-Cy5.5. Robust accumulation of AnxCLIO-Cy5.5 is seen throughout the midmyocardium (yellow arrows). In contrast, only small foci of hypointensity from the persistence of Inact_CLIO-Cy5.5 are seen. E, Fluorescence microscopy (magnification ×100) of AnxCLIO-Cy5.5 uptake correlates well with the in vivo MR images (A and B). The endocardial boundary in E has been manually traced to aid visualization (white arrows indicate epicardium). F, Fluorescence microscopy (×400) shows AnxCLIO-Cy5.5 bound to the cell surface of morphologically intact CMs.
apoptosis develops most frequently in the midmyocardium, whereas CM necrosis appears earliest in the subendocardium. We show, moreover, that the majority of apoptotic CMs within 4 to 6 hours of ischemia-reperfusion remain potentially viable and provide an important potential target for myocardial salvage.

Annexin-V binds to phosphatidylserine on the outer surface of apoptotic CMs but can also bind to phosphatidylserine. Figure 5. Predominance of CM apoptosis and AnxCLIO-Cy5.5 accumulation in the midmyocardium. A, Fluorescence microscopy of AnxCLIO-Cy5.5 (magnification ×200) shows the agent bound to CMs in the midmyocardium with sparing of the subendocardium (asterisk marks subendo/midmyocardial boundary). B, TUNEL staining (×400) of the midmyocardium shows the presence of numerous apoptotic CMs. C, Immunohistochemistry for NBD (×200) shows no evidence of Gd-DTPA-NBD accumulation in either the subendocardium or midmyocardium. D, Fluorescence microscopy (×100) of the mid and subepicardium shows robust accumulation of AnxCLIO-Cy5.5 in the midmyocardium but not in the subepicardium (asterisk marks midmyo/subepicardial boundary). E, TUNEL-positive CMs (×400). Arrow points to a TUNEL-positive interstitial cell (Int) in the field. The majority of the TUNEL-positive cells taking up AnxCLIO-Cy5.5 are CMs.

Figure 6. Molecular MRI of CM apoptosis (A, D, and G) and simultaneous DE MRI of Gd-DTPA-NBD (B, E, and H) in a mouse with severe injury. Images at 3 slice locations are shown, moving progressively from the midventricular level (A and B) to the left ventricular apex (G and H). B, At the midventricular level, only a small area in the subendocardium of the lateral wall shows DE (red arrows). E and H, The extent of DE increases progressively in the more apical slices (red arrows) and is fairly extensive at the apex. Although the accumulation of AnxCLIO-Cy5.5 is fairly transmural, DE of Gd-DTPA-NBD is seen predominantly in the subendocardium. C, F, and I, Immunohistochemistry for Gd-DTPA-NBD confirming the in vivo MRI findings. C, Control area in the uninjured septum showing no evidence of DE (magnification ×200). F (×200) and I (×400), Sections from the antero-apical wall of the left ventricle show positive staining for Gd-DTPA-NBD in areas of the subendocardium with significant amounts of CM degeneration.
Propidium iodide, which cannot cross the intact cell membrane, is thus used in vitro in conjunction with annexin as a marker of cell rupture and necrosis. The clinical studies performed to date, however, have used 99mTc-annexin in isolation or in combination with perfusion imaging and, although of significant value, were not able to distinguish CM apoptosis from CM necrosis. The dual-contrast molecular MRI approach presented here is conceptually analogous to the in vitro use of fluorescent annexin-V and propidium iodide but is, in addition, highly amenable to noninvasive imaging and clinical translation.

Gadolinium chelates do not cross intact cell membranes, and DE of gadolinium in acute injury is thus indicative of its accumulation in nonviable areas of CM necrosis. The use of Gd-DTPA-NBD allowed the presence of DE to be confirmed histologically, providing a novel tool to validate the accuracy of the presented dual-contrast approach. NBD, unlike most fluorochromes, is a small molecule with no charge and minimal potential to bind to albumin and other macromolecules. These properties ensured that the kinetics of Gd-DTPA-NBD remained similar to those of Gd-DTPA and suitable for DE imaging. Initial testing in 2 infarcted mice revealed that freeze artifact in the study was not prevalent or problematic.

At high field strengths, the R2/R1 ratio of AnxCLIO-Cy5.5 approaches 80, and the use of a TE of 1 ms thus produces a proton density–weighted image (Figure 1). The R2/R1 ratio of small gadolinium chelates, although higher at 9.4 T than at clinical field strengths, is significantly lower than that of AnxCLIO-Cy5.5. The R1 effects of small gadolinium chelates at 9.4 T thus still dominate at a TE of 1 ms, producing signal enhancement. At clinical field strengths, because the R2/R1 ratio of iron oxide decreases significantly, a longer TE is needed to balance its R2 and R1 effects. A TE of 9 ms, for instance, was used in an angiography study at 1.5 T to eliminate signal enhancement from iron oxide while still producing a T1 bright signal from gadolinium. The use of a longer TE to decouple the effects of iron oxide and gadolinium at clinical field strengths is well tolerated because the R2/R1 ratio of gadolinium is lower at clinical fields than at 9.4 T. Off resonance, magnetization transfer and other novel sequences probably will provide additional mechanisms for dual-contrast imaging. The use of a dual-contrast approach in selected clinical settings thus seems highly feasible but will require further study.
may be particularly vulnerable to ischemic injury in animals with a paucity of preformed collateral networks. Further study will be needed to determine the mechanisms underlying this spatial pattern of apoptosis and whether it is replicated in larger animals and humans. Strategies to salvage high numbers of apoptotic CMs in the midmyocardium, however, could potentially transform a highly transmural insult into a significantly better tolerated subendocardial infarct.24,25

The absence of delayed gadolinium enhancement in areas of severe microvascular destruction has been well described.26,27 Because AnxCLIO-Cy5.5 is >1000 times larger than Gd-DTPA-NBD,10 delivery of this agent through the microvasculature is likely to become impaired well before that of Gd-DTPA-NBD. The presence of small foci in the subendocardium showing DE of Gd-DTPA-NBD but no accumulation of AnxCLIO-Cy5.5 probably can be accounted for on this basis. Overall, however, the results of this study show that, within 4 to 6 hours of ischemia-reperfusion, the majority (>70%) of injured myocardium is characterized only by the accumulation of AnxCLIO-Cy5.5 (Figure 7). This result, consistent with prior pathological studies,28 suggests that large numbers of viable and potentially salvageable apoptotic CMs may be present in the myocardium within 4 to 6 hours of ischemia-reperfusion.

Loss of segmental function in the study was associated with transmural accumulation of AnxCLIO-Cy5.5 and the presence of DE (Figure 7). Further study will be needed to determine the contribution of myocardial stunning and the potential of antiapoptotic strategies to improve long-term contractility. The potential of annexin-positive CMs to be salvaged is supported by a recent study involving 9 patients with acute coronary syndromes.15 The extent of 99mTc-annexin uptake was imaged by SPECT within 24 hours of the ischemic event and found to be larger than the perfusion defect in the healed infarct.15 SPECT imaging has also been used in a dual-contrast approach in rats with ischemia-reperfusion.29 99mTc-annexin-V was cojected with an indium-labeled antimyosin antibody, which can only bind to myosin when cell rupture/necrosis occurs. CM apoptosis was predominant within the first 4 hours of reperfusion, at which time CM necrosis began to be detected as well.29 Translation of this dual SPECT approach is feasible but would involve a high dose of radiation and produce isolated SPECT hotspots. Moreover, this approach would still have low spatial resolution and the dynamic range limitations (high background signal) seen in prior SPECT studies of 99mTc-annexin.3,15,16

The kinetics of AnxCLIO-Cy5.5 and Inact_CLIO-Cy5.5 within the first few hours of ischemia and probe injection are complex and are influenced significantly by the extent and severity of myocardial injury. Limitations of the dual-contrast technique also must be considered. The use of a longer TE in the presence of significant probe accumulation can reduce specificity and lead to T2* blooming. Further study will thus be needed, under a range of conditions, to determine the optimal TE and dose of AnxCLIO-Cy5.5 to use. AnxCLIO-Cy5.5 accumulates principally on the outer surface of apoptotic CMs,30 with probe accumulation driven by a natural microenvironment. Decoupling the effects of iron oxide and gadolinium will be more difficult when artificially high local concentrations of iron oxide are encountered, such as those produced by intramyocardial injection of iron oxide–labeled stem cells.

In conclusion, a novel dual-contrast molecular MRI approach to image CM apoptosis and necrosis is presented. We show with this approach that CM apoptosis can be imaged in vivo with high specificity, high spatial resolution, and within the first 4 to 6 hours of ischemia, during which apoptosis is most prevalent. Although some loss of CM viability occurs within the first few hours of ischemia-reperfusion, the results of this study suggest that the majority of apoptotic CMs, particularly those in the midmyocardium, remain viable within 4 to 6 hours of ischemia-reperfusion. These apoptotic but viable midmyocardial CMs thus form an attractive target for potential myocardial salvage and offer the potential of converting a large transmural injury into a significantly better-tolerated subendocardial infarct.

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Disclosures

None.

References

CLINICAL PERSPECTIVE

Apoptosis is the predominant mechanism of cardiomyocyte (CM) death during the first 4 to 6 hours of ischemia. Molecular imaging of CM death, however, has previously been performed 12 to 24 hours after injury, and the techniques used were not able to distinguish CM apoptosis from CM necrosis. In this report, we describe a dual-contrast molecular MRI approach that allows CM apoptosis to be imaged within the first 4 hours of ischemia and to be distinguished from CM necrosis. Areas of CM apoptosis accumulate AnxCtIO-Cy5.5 but do not show delayed enhancement of Gd-DTPA-NBD. Necrotic myocardium, however, accumulates both probes. In a mouse model of ischemia-reperfusion, we show that CM apoptosis is most frequent in the midmyocardium, whereas CM necrosis develops first in the subendocardium. Within 4 to 6 hours of ischemia-reperfusion, however, >70% of apoptotic CMs did not show delayed enhancement of Gd-DTPA-NBD and were thus potentially viable. The described dual-contrast molecular MRI approach is highly translatable and of high clinical relevance. Our study suggests that numerous apoptotic but potentially salvageable CMs are present in the midmyocardium in the first few hours of ischemic injury. Salvage of these midmyocardial apoptotic CMs could convert a potentially transmural insult into a better-tolerated subendocardial infarct. Further study will be needed to determine whether similar patterns of CM apoptosis are seen in humans. The presence of numerous apoptotic CMs in the midmyocardium, however, would constitute a highly important and attractive target for potential myocardial salvage.
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