Fluorescence Tomography and Magnetic Resonance Imaging of Myocardial Macrophage Infiltration in Infarcted Myocardium In Vivo

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Background—Fluorescence imaging of the heart is currently limited to invasive ex vivo or in vitro applications. We hypothesized that the adaptation of advanced transillumination and tomographic techniques would allow noninvasive fluorescence images of the heart to be acquired in vivo and be coregistered with in vivo cardiac magnetic resonance images.

Methods and Results—The uptake of the magnetofluorescent nanoparticle CLIO-Cy5.5 by macrophages in infarcted myocardium was studied. Ligation of the left coronary artery was performed in 12 mice and sham surgery in 7. The mice were injected, 48 hours after surgery, with 3 to 20 mg of iron per kilogram of CLIO-Cy5.5. Magnetic resonance imaging and fluorescence molecular tomography were performed 48 hours later. An increase in magnetic resonance imaging contrast-to-noise ratio, indicative of myocardial probe accumulation, was seen in the anterolateral walls of the infarcted mice but not in the sham-operated mice (23.0 ± 2.7 versus 5.4 ± 2.4; P < 0.01). Fluorescence intensity over the heart was also significantly greater in the fluorescence molecular tomography images of the infarcted mice (19.1 ± 5.2 versus 5.3 ± 1.4; P < 0.05). The uptake of CLIO-Cy5.5 by macrophages infiltrating the infarcted myocardium was confirmed by fluorescence microscopy and immunohistochemistry.

Conclusions—Noninvasive imaging of myocardial macrophage infiltration has been shown to be possible by both fluorescence tomography and magnetic resonance imaging. This could be of significant value in both the research and clinical settings. The techniques developed could also be used to image other existing fluorescent and magnetofluorescent probes and could significantly expand the role of fluorescence imaging in the heart. (Circulation. 2007;115:1384-1391.)

Key Words: fluorescence ■ inflammation ■ magnetic resonance imaging ■ myocardium ■ tomography

Fluorescence-based imaging techniques have become an integral component of modern cardiovascular research. However, with the exception of a few landmark studies using intravital microscopy or photographic imaging of the exposed heart,1–3 cardiac fluorescence imaging has generally been limited to ex vivo and in vitro applications.4,5 In the present study, we describe the application of advanced transillumination and tomographic techniques to allow fluorescence imaging of the heart to be performed noninvasively in vivo. In particular, deep-seated fluorescence activity in the heart is resolved with the use of fluorescence molecular tomography (FMT), a technique developed for 3-dimensional and quantitative fluorescence imaging.6,7

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The feasibility and value of cardiac FMT are demonstrated in the present study in a murine model of postinfarction myocardial macrophage infiltration. Postinfarction injury is, at present, often assessed by the retention of gadolinium within the myocardium by magnetic resonance imaging (MRI).8 MRI agents targeted to specific molecular processes such as cardiomyocyte apoptosis have also been developed recently.5 Cardiac MRI thus allows comprehensive anatomic, functional, and molecular imaging of the myocardium to be performed. Nevertheless, the combination of MRI with fluorescence imaging is highly desirable because fluorescence imaging offers sensitive detection of fluorochromes at picomole amounts (nanomolar concentrations)7,9 and allows multispectral imaging of several biological processes to be performed simultaneously with high throughput.7,9 These attributes complement those of MRI such as its high spatial resolution, excellent soft tissue contrast, and ability to characterize myocardial contraction and function.

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The magnetofluorescent nanoparticle (MNP) CLIO-Cy5.5 was used in the present study to provide dual magnetic and fluorescence readouts of postinfarction myocardial macrophage infiltration and to demonstrate the synergy and congruence of this dual-modality approach. MNPs are avidly taken up by macrophages and have been used previously to image tissue inflammation in diabetic insulitis, atherosclerosis, encephalitis, and cardiac transplant rejection. We show in the present study that the MNP CLIO-Cy5.5 can be used to image postinfarction myocardial macrophage infiltration by both MRI and FMT. Although FMT has been used previously to image tumors and atherosclerosis in mice, this, to the best of our knowledge, is the first successful application of FMT in the heart. Several of the techniques described in the present study have the potential for translation and could thus be of significant value in both research and clinical settings.

**Methods**

**Image Acquisition**

Myocardial infarction was induced in 12 C57Bl/6 mice by complete ligation of the left coronary artery under isoflurane anesthesia. The surgery was performed via anterior sternotomy in a first series of 7 mice and via lateral thoracotomy in a second series of 5 mice. Sham surgery was performed in 7 mice via the sternotomy approach and consisted of visualization of the left coronary artery without ligation. All animal experiments were performed in accordance with the guidelines for research animal care at our institution.

The 7 infarcted mice in series 1 and the 7 sham-operated mice all received a tail vein injection of the MNP CLIO-Cy5.5 (20 mg iron per kilogram) 48 hours after infarction. The 5 infarcted mice in series 2 were injected with varying amounts of CLIO-Cy5.5 to determine the dose response of both the MRI and fluorescence signals and their correlation with each other. Two of the mice in series 2 received 20 mg iron per kilogram, 2 received 10 mg iron per kilogram, and 1 received 3 mg iron per kilogram. The blood half-life of CLIO-Cy5.5 in mice is ~10 hours, and in vivo imaging in all mice was thus performed 48 hours after injection (96 hours after the infarct). This allowed for almost complete clearance of any unbound probe and also allowed sufficient time for the nanoparticle to be taken up by macrophages infiltrating the healing infarct.

In vivo MRI was performed on 3 infarcted mice in series 1, 3 sham-operated mice, and all 5 infarcted mice in series 2. Imaging was performed on a 9.4-T horizontal-bore small-animal scanner (Biospec Bruker, Billerica, Mass) with dual ECG and respiratory gating (SA Instruments, Stonybrook, NY). Localizer scans were performed to identify the short axis of the left ventricle, after which conventional fast low-angle shot (FLASH) cine were performed at several short-axis locations to examine myocardial function. Parameters for these FLASH cine included the following: field of view 30 mm, slice 1 mm, matrix 200×200, flip angle 30 degrees, 16 frames per RR interval, echo time 2.7 ms, 4 averages. A single short-axis slice location, based on the presence of extensive hypokinesis in the anterior and lateral walls, was then chosen for further analysis. T2*-weighted susceptibility imaging was performed at this location with a modified FLASH cine sequence, which incorporated first-order gradient moment nulling in all directions and contained only 6 frames per RR interval. T2*-weighted images were acquired at echo times of 3.5 and 6.5 ms.

After MRI, the mice underwent fluorescence imaging in vivo (infarcted mice, n = 12; sham mice, n = 5) under isoflurane anesthesia. Sequential MRI and FMT acquisitions were thus acquired in 8 of 12 infarcted mice (3 in series 1 and 5 in series 2). Fluorescence imaging was performed with the use of a modular home-built scanner capable of acquiring transillumination, reflectance, and absorption data at multiple projections needed for tomographic imaging. An excitation laser diode at 672 nm, tailored to the imaging of Cy5.5, was used. The excitation system consisted of 46 fibers spread over a 20×20 matrix in a slab geometry imaging chamber. Each of the 46 excitation sources was illuminated individually over a discrete 5-second time gate, yielding a total acquisition time of ~7 minutes per mouse. For each fiber, 4 different sets of data were acquired (intrinsinc fluorescence, extrinsic fluorescence, and intrinsic and fluorescence noise) with the use of a 512×512-element, ultralow-noise, cooled charge-coupled device (CCD) camera (model 7471; Roper Scientific, Trenton, NJ). Each of the fibers effectively established a different projection through the animal over a limited projection angle in analogy to x-ray tomosynthesis.

The mice were partially immersed in a scattering medium consisting of 1% Intralipid and 150 ppm of india ink to simplify experimental issues. In particular, this matching medium sufficiently attenuates stray light from the fibers or minimally attenuated light from the side boundaries of the animal from hitting the CCD camera. In this way, the dynamic range of the measurements is better interfaced to the dynamic range of the CCD camera used for detection.

**Image Processing**

For each imaging study, the in vivo optical measurements were processed in 4 different ways, as follows.

A transillumination intrinsic image (TMI) was produced by summing all 46 images collected at the excitation wavelength. Background noise was rejected by applying a threshold equal to 20 times the standard deviation of the background noise on each image before summation. The TMI are essentially attenuation maps of light intensity by tissue structures and were used to guide subsequent analysis and reconstruction of the fluorescence images. Regions in the thorax with absorption values >3-fold the background tissue attenuation were found to correspond to the heart, whereas a 6-fold increase in attenuation in the abdomen was congruent with the liver.

The second processing method calculated the transillumination ratio image (TRI) by first dividing each fluorescence image with the corresponding image collected at the excitation wavelength and then summing all of the 46 resulting ratio images. Background subtraction was performed as previously described.

The third technique involved the acquisition of an additional background image for each source employed, collected through the matching medium at the emission wavelength with the mouse removed. The background image was used to calculate the attenuation-corrected image (ACI) by dividing each fluorescence image by an attenuation-corrected ratio (image collected through mouse at the excitation wavelength/background image), and then summing all of the 46 processed images. This operation is similar to the one used to derive the TRI images but uses a corrected attenuation ratio to divide the fluorescence measurements. The operation used in the ACIs is thus better able to compensate for the effects of spatially heterogeneous tissue attenuation because it is less sensitive to contrast loss farther away from the illuminating source.

The fourth processing method treated the data as tomographic projections and utilized FMT algorithms for reconstructing depth-resolved images. The tomographic particulars have been well described, and an analytical presentation is beyond the scope of this report. In brief, the method uses a physical model of photon propagation based on the diffusion equation to predict photon propagation in tissues for each source and CCD camera measurement used. This description of photon propagation is formulated as a linear algebraic problem that on solution (inversion) yields the unknown 3-dimensional image of fluorochrome biodistribution. Importantly, this solution inherently compensates for spatial variations in tissue attenuation. Modulation of the FMT images by an attenuation correction factor is thus not necessary.

The performance of the fluorescence imaging system and inversion techniques used in the present study has been studied extensively. The technique has been shown to be capable of accurately resolving fluorescence signals from superficial or deep-seated structures even in the presence of large spatial variations in attenuation, such as those expected between the myocardium, blood-filled ventricles, lungs, and bone.

Coronal tomographic reconstructions were performed in the present study to allow the resulting individual 2-dimensional slices to be...
correlated with the absorption maps (TMIs), which were also acquired in a coronal orientation. Eight coronal (z-direction resolved) 2-dimensional slices were thus reconstructed over a 12-mm volume, producing reconstructed voxel dimensions (xyz) of 1.8 x 1.8 x 1.5 mm. Submillimeter voxel resolution, however, has been achieved previously with this system. 18

Signal intensity in the FMT data set was measured in a volume, defined in 2 dimensions by the cardiac silhouette on the planar absorption maps and in the third dimension by the reconstructed tomographic slices. Three adjacent tomographic slices in the reconstructed coronal data set were judged to intersect the heart (slices 2 to 4). The slices on either side of this, which passed either anterior (slice 1) or posterior (slices 5 to 8) to the heart, could thus be excluded from the quantitative analysis. The fluorescence signal in a 2-dimensional region, defined by the cardiac silhouette on the absorption map, was measured in each of the 3 tomographic slices known to intersect the heart and summed to yield the total cardiac fluorescence signal in each mouse.

Fluorescence Microscopy and Histology
After FMT, the mice were euthanized, and the hearts were excised for fluorescence reflectance imaging (FRI), fluorescence microscopy, and histology (infarcted mice, n=5; sham-operated mice, n=5). FRI was performed with a 12-bit CCD camera (Kodak, Rochester, NY), with the use of a dedicated Cy 5.5 bandpass emission filter (680 to 720 nm; Omega Optical, Brattleboro, VT) and an exposure time of 120 seconds. Fluorescence intensity was measured in the apical half of the ventricle, corresponding to the location of the infarcts. After FRI, the hearts were embedded and sectioned for fluorescence microscopy and histology.

Fluorescence microscopy was performed with the use of an upright epifluorescence microscope (Eclipse 80i; Nikon Instruments, Melville, NY) with a cooled CCD camera (Cascade; Photometrics). Fluorescence images were obtained with the following filters and parameters: excitation 650 nm, emission 680 nm longpass and 710 ± 25 nm bandpass, exposure time 120 seconds. Immunohistochemistry for macrophages was performed with the use of a rat anti-Mac3 monoclonal antibody (Pharmingen, San Diego, Calif). An avidin-biotin peroxidase method was used, and the reaction was visualized with 3-amin-9-ethyl-carbazol as substrate (Sigma Chemical, St Louis, Mo), yielding a red reaction product in the cytoplasm. Nuclei were counterstained with Mayer’s hematoxylin solution (Sigma Chemical). Adjacent sections treated with nonimmune IgG-provided controls for antibody specificity.

The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

Results
MRI of the infarcted mice consistently revealed the presence of left ventricular dilatation with thinning and akinesis of the anterior, lateral, and inferolateral walls of the left ventricle (Figure 1). The presence of negative contrast enhancement, consistent with the accumulation of the magnetic nanoparticle, could be seen clearly in the hypocontractile areas of myocardium particularly at an echo time of 6.5 ms (Figure 1). No evidence of nanoparticle accumulation, however, was seen by MRI in the hearts of the sham-operated mice.

The contrast-to-noise ratio (CNR) between the uninjured septal myocardium and the hypocontractile anterolateral wall was measured in the MR images. CNR values in the series-1 infarcted mice and the sham-operated mice were then compared with an unpaired t test (GraphPad-Prism). The average CNR in the infarcted mice was 23.0 ± 2.7, whereas in the sham mice it was 5.43 ± 2.4 (P < 0.01) (Figure 1).

The accumulation of CLIO-Cy5.5 in the infarcted myocardium could also be seen clearly by fluorescence imaging. Two areas of elevated fluorescence intensity were seen on the planar images of the infarcted mice in vivo. Correlation with the absorption maps revealed consistently that the lower of these 2 foci corresponded to the hepatic uptake of CLIO-Cy5.5, whereas the upper focus fell over the heart (Figure 2). An equivalent amount of hepatic signal was seen in the

Figure 1. Imaging of myocardial macrophage infiltration in vivo by MRI. Midsystolic images of an infarcted mouse (A to C) and a sham-operated mouse (D, E) are shown. The heart of the infarcted mouse is dilated with profound thinning and hypokinesis of the anterior and lateral walls. The accumulation of the iron-oxide probe in the injured anterolateral myocardium (arrows) can be seen clearly as the echo time is increased from 2.7 ms (A) to 3.5 ms (B) and 6.5 ms (C). No evidence of probe accumulation is seen in the sham-operated mouse at echo times of 3.5 ms (D) and 6.5 ms (E). The CNR between the septum and anterolateral wall was significantly greater (*P < 0.01) in the infarcted mice than the sham-operated mice (F).

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sham-operated mice, but a significantly lower amount of signal was seen over the heart and thorax (Figure 2).

The differences in fluorescence intensity between the infarcted and sham-operated mice were evident on both TRI and ACI (Figure 2). The heart, however, was more clearly delineated on ACI for the reasons described in the aforementioned methods. Normalized thoracic fluorescence intensity (thoracic/abdominal fluorescence) was significantly greater in the infarcted than the sham-operated mice (Figure 3). Normalized thoracic fluorescence in the infarcted and sham-operated mice was 0.58±0.09 versus 0.28±0.03 by TRI (P<0.05) and 0.57±0.1 versus 0.28±0.04 by ACI (P<0.05).

FMT imaging further demonstrated the accumulation of the probe in the hearts of the infarcted mice but not in the hearts of the sham-operated mice (Figure 4). Importantly, the availability of 3-dimensional tomographic data sets and absorption maps of the heart allowed fluorescence intensity to be measured in a region of interest defined by the true volume of the heart. Mean fluorescence intensity (arbitrary units) in this volume was 19.1±5.2 in the series-1 infarcted mice versus 5.3±1.4 in the sham mice (P<0.05).

The findings of the in vivo MRI and fluorescence imaging were further confirmed by ex vivo FRI and microscopy. Fluorescence intensity by FRI was significantly higher in the infarcted hearts than in the sham-operated hearts, at 125.0±30.6 versus 45.2±6.5 (P<0.05), as shown in Figure 5. Histology of the excised hearts consistently revealed the infiltration of inflammatory cells into the infarcted myocardium on hematoxylin-eosin staining (Figure 6A). Near-infrared fluorescence microscopy showed a significant increase in signal intensity only in those areas of the myocardium with an inflammatory infiltrate (Figure 6B). Immunohistochemical staining with the Mac3 antigen confirmed the presence of numerous macrophages within the inflammatory cell infiltrate (Figure 6C to 6D). A very high degree of colocalization was seen consistently between areas

Figure 2. Planar in vivo fluorescence images of an infarcted mouse (A to D) and a sham-operated mouse (E to H). The fluorescence images have been superimposed on white light (WL) images of the mice (A, E). The anatomic silhouette of the heart (arrows) and the triangular-shaped liver below it can be identified on the TMI (B, F), which are essentially near-infrared absorption maps. The use of these absorption maps allowed both the planar and subsequent tomographic fluorescence images to be coregistered with an anatomic image of the heart. The larger silhouette of the infarcted than the sham-operated heart on the absorption maps (TMI) likely represents the increased volume of blood in the dilated infarcted left ventricle. Shown also are the TRI (C, G) and the ACI (D, H). Significantly greater fluorescence signal was seen over the hearts of the infarcted mice (B through D) than the sham-operated mice (F through H).

Figure 3. Thoracic fluorescence intensity on the TRI (A) and the ACI (B). Thoracic fluorescence has been normalized by the corresponding value in the abdomen to yield a normalized thoracic fluorescence (NTF). Significantly greater (*P<0.05) thoracic fluorescence was seen in the infarcted mice than in the sham-operated mice.
of elevated fluorescence intensity by fluorescence microscopy and areas of positive macrophage staining by immunohistochemistry. This finding is consistent with the known uptake of CLIO-Cy5.5 by tissue macrophages.10,12,14

MRI of the 5 infarcted mice in series 2 revealed the presence of negative contrast enhancement in the anterolateral myocardium of all mice. A clear increase in the CNR between the anterolateral and septal walls was seen as the dose of CLIO-Cy5.5 was increased (Figure 7). Likewise, a clear increase in fluorescence intensity over the heart was seen in both the planar and FMT images as the dose of CLIO-Cy5.5 was increased. The CNR and FMT signals were both normalized by the highest respective values in their data sets and thus scaled from 0 to 1. A strong correlation \( r^2=0.83, P<0.05 \) between CNR by MRI and cardiac fluorescence intensity by FMT was seen (Figure 7).

**Discussion**

Molecular imaging of apoptosis,5,24,25 transglutaminase activity,26 and matrix metalloproteinase activity27 in the myocardium has been performed previously in vivo with the use of single photon emission computed tomography and MRI. Fluorescence imaging of the myocardium, however, has been limited largely by the inability to perform this noninvasively in vivo. In the present study, we report the development and successful application of transillumination-based methods, which include FMT, for the noninvasive imaging of the mouse heart in vivo. These transillumination and tomographic fluorescence techniques not only allowed myocardial macrophage infiltration to be imaged successfully in vivo in the present study but also pave the way for the future imaging of targeted and activatable fluorochromes in the myocardium.2,4,28

Fluorescence tomography of subsurface structures, such as the heart, is best performed in the near-infrared range, where the absorption of light by endogenous pigments and tissue autofluorescence are both lowest.6,29 However, even within this confined range, multiwavelength tomographic imaging remains possible and has been performed successfully in vivo.9 Tomographic fluorescence imaging of the myocardium is complicated by several additional factors, which include the presence of extremely heterogeneous tissues within the thorax and the high concentration of hemoglobin in the chambers of the heart. However, we have shown previously that the tomographic methodology used in the present study has a low sensitivity to variations in hemoglobin concentra-

![Figure 4. FMT of myocardial macrophage infiltration in vivo. Reconstructed coronal slices from the 3-dimensional FMT data set have been superimposed on white light images of the mice. Slices 2 to 4 in the FMT data set intersected the heart, slice 1 passed anterior to it, and slices 5 to 8 passed posterior to it. A, Long-axis MRI slice in an infarcted mouse corresponding to slice 2 from the fluorescence data set of that mouse (B). C, Slice 5 from the fluorescence data set of the infarcted mouse. The corresponding slices (D=slice 2, E=slice 5) of a sham-operated mouse are shown. Fluorescence intensity over the heart was significantly greater (*P<0.05) in the infarcted mice than the sham-operated mice (F).](image-url)

![Figure 5. FRI of excised mouse hearts ex vivo. Shown are images of an infarcted mouse heart (A) and images of a sham-operated mouse (B). Fluorescence intensity in the infarcted mouse hearts was significantly greater (*P<0.05) than in the sham-operated mice (C).](image-url)
tion and other sources of optical signal attenuation.\textsuperscript{22,23} The similarity in the relative signal intensities recorded from the infarcted and sham hearts by in vivo FMT and ex vivo FRI in the present study (Figures 4 and 5) supports these prior observations.

The high hemoglobin concentration in the chambers of the heart allows an anatomic silhouette of the heart to be defined on an absorption map of the thorax and to be coregistered with the fluorescence molecular images. Alternative approaches for anatomic coregistration include the use of a second intravascular fluorochrome to delineate the chambers of heart or coregistration of the fluorescence data set with a second imaging modality such as MRI, as shown in Figure 4.

Coregistration of fluorescence tomographic data with MRI,\textsuperscript{28} however, not only exploits the dual-modality nature of MNPs such as CLIO-Cy5.5 but is also particularly complementary in the myocardium. The high sensitivity and multispectral nature of fluorescence imaging complement the high spatial resolution of MRI and its ability to evaluate myocardial perfusion, viability, mechanics, and contractile function.

The accumulation of CLIO-Cy5.5 in the myocardium could be seen in the present study on both the transillumination and tomographic fluorescence images (Figures 2 and 4). However, the transillumination images were only resolved in 2 dimensions, and the possibility of signal from tissues lying either directly anterior or posterior to the heart contributing to

\begin{figure}[h]
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\includegraphics[width=\textwidth]{figure6}
\caption{Uptake of the magnetofluorescent contrast agent CLIO-Cy5.5 by macrophages infiltrating the healing infarct. The images shown are as follows: hematoxylin-eosin section of infarct territory (A), fluorescence microscopy (B), and immunohistochemistry for the Mac3 macrophage antigen (C, D). The area of myocardium shown in B and C is demarcated by the dark black lines in panel A. A magnified view of the region marked by the dark arrow in C is shown in D. The fluorescence signal from the probe colocalized extremely well with the macrophage infiltrate, consistent with the uptake of CLIO-Cy5.5 by the infiltrating macrophages.}
\end{figure}

\begin{figure}[h]
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\includegraphics[width=\textwidth]{figure7}
\caption{Correlation of myocardial CNR by MRI and cardiac fluorescence intensity by FMT. MR and FMT images were acquired 48 hours after the injection of CLIO-Cy5.5 in doses ranging from 3 to 20 mg iron per kilogram. A to C, T2- weighted MR images at an echo time of 6.5 ms in mice injected with 3 mg iron per kilogram (A), 10 mg iron per kilogram (B), and 20 mg iron per kilogram of CLIO-Cy5.5 (C). A progressive increase in negative contrast enhancement and CNR in the injured myocardium (arrows) is seen as the dose of injected CLIO-Cy5.5 is increased. D, A high degree of correlation is seen between the normalized CNR by MRI and the normalized cardiac fluorescence intensity by FMT ($r^2=0.83$, $P<0.05$).}
\end{figure}
the apparent cardiac signal could thus not be excluded. The ability to resolve the tomographic fluorescence data in all 3 dimensions allowed the fluorescence signal to be analyzed in a volume corresponding to the heart only and prevented signal from adjacent tissues such as the chest wall from potentially influencing the data.

Fluorescence microscopy and immunohistochemistry confirmed the uptake of CLIO-Cy5.5 by macrophages infiltrating the infarcted tissue (Figure 6). The pathophysiological significance of such macrophage infiltration in the postinfarction setting, however, remains incompletely understood.30 Tissue macrophages may play a vital role in the removal of necrotic tissue and the creation of new scar tissue.30–33 On the other hand, these cells may contribute to myocardial remodeling via the release of matrix-degrading enzymes and also produce excessive myocardial fibrosis.30,34,35 In addition, animal studies using either chemokine or antichemokine therapy32–34,36,37 have shown conflicting results. The ability of molecular imaging techniques to image myocardial macrophage infiltration in vivo could provide valuable insights into the role of these cells in the finely balanced inflammatory milieu of a healing infarct.

A high correlation between CNR by MRI and cardiac fluorescence signal intensity was seen in the present study, and both techniques showed a clear dose response (Figure 7). Individual differences in the response to cytokine therapy in the postinfarction setting have been reported recently,38 and magnetofluorescent imaging of myocardial macrophage infiltration in vivo could thus allow the timing, dose, and efficacy of a particular therapy to be quantitatively assessed and individualized. The prolonged retention of internalized MNPs by macrophages, however, may limit the utility of these nanoparticles for repeated serial imaging of myocardial macrophage infiltration.

Noninvasive fluorescence imaging is currently well suited for cardiac imaging in mice and potentially in rats up to a depth of 4 to 5 cm. Whole-body optical imaging would be significantly more challenging in humans, however, because of the need for photons to penetrate deeper than a few centimeters into cardiac tissue. In these cases, catheter-based or minimally invasive approaches may be needed and are highly feasible.

MNPs such as CLIO-Cy5.5 therefore have significant potential for future translation. The MNP Combidex (analogous to CLIO) has completed phase 3 clinical trials.39 Likewise, the near-infrared fluorochrome indocyanine green (analogous to Cy5.5) has been used clinically for many years.39 Magnetofluorescent contrast agents thus have the potential to play a central role in the molecular imaging of the cardiovascular system in both research and clinical settings.

Conclusion

The imaging of myocardial macrophage infiltration in vivo has been shown in the present study to be possible by both FMT and MRI, and analogues of the probe used have already been used extensively in humans. The imaging of myocardial macrophage infiltration in vivo has the potential to be of significant value in both research and clinical settings and may play an important role in the study of postinfarction healing as well as other conditions such as myocarditis, heart failure, and transplant rejection.

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Disclosures

None.

References


**CLINICAL PERSPECTIVE**

The ability to noninvasively image cellular and subcellular events in the complex milieu of the healing myocardial infarct could facilitate the development of novel cardioprotective therapies. In this article, we report the use of a dual-modality (magnetic and fluorescent) nanoparticle to image myocardial macrophage infiltration in healing myocardial infarcts. Magnetic resonance imaging and a novel fluorescence tomographic imaging technique (fluorescence molecular tomography) are used to image the uptake of a macrophage-avid nanoparticle in infarcted myocardium. The techniques described in the study have the potential to affect the care of the postinfarction patient both directly and indirectly. Analogues of the nanoparticle used in the present study have completed phase 3 clinical trials. In the near future, myocardial macrophage infiltration in the healing infarct could therefore potentially be imaged by magnetic resonance imaging or fluorescence techniques in the appropriate clinical setting. Imaging myocardial macrophage infiltration could prove extremely useful in guiding, for instance, the use of either procytokine or anticytokine therapy in selected postinfarction patients. Clinical trials of these therapies to date have been inconsistent, perhaps in part because of the inability to image myocardial macrophage levels on an individual basis. The synergy and value of a dual-modality (magnetic resonance imaging and fluorescence) strategy for myocardial imaging are also demonstrated in the article as a powerful adjunct to multiparametric magnetic resonance imaging technologies.
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