Discrimination of Myocardial Acute and Chronic (Scar) Infarctions on Delayed Contrast Enhanced Magnetic Resonance Imaging With Intravascular Magnetic Resonance Contrast Media

Maythem Saeed, DVM, PhD,* Oliver Weber, PhD,* Randall Lee, MD, PhD,† Loi Do, BS,* Alastair Martin, PhD,* David Saloner, PhD,* Philip Ursell, MD,‡ Philippe Robert, PhD,§ Claire Corot, PhD,§ Charles B. Higgins, MD, FACC*

San Francisco, California, and Paris, France

OBJECTIVES
The purpose of this study was to examine the potential of intravascular gadolinium (Gd)-chelates in discriminating acute from chronic myocardial infarctions (MIs).

BACKGROUND
A potential limitation of delayed contrast enhanced magnetic resonance imaging with standard extracellular Gd-chelates is its inability to distinguish acute from chronic MIs.

METHODS
Eight pigs with MIs were studied at 3 days and 8 weeks. Inversion recovery gradient echo (IR-GRE), T1-turbo spin echo (TSE), and T2-TSE images were acquired before and after administration of intravascular and extracellular Gd-chelates. Triphenyltetrazolium chloride (TTC) was used to delineate infarctions at postmortem. Masson’s trichrome and Biotinylated Bandeiria simplicifolia Isolectin B4 stains were used to characterize scarred myocardium. Analysis of variance was used to compare signal intensity (SI) ratios and determine differences in infarct extent.

RESULTS
The intravascular agent produced differential enhancement of acute infarctions at 3 days (SI ratio 5.8 ± 1.3) but not at 8 weeks (1.6 ± 0.4, p < 0.01). The extracellular agent provided differential enhancement of both acute (SI ratio 7.7 ± 1.4) and chronic (7.5 ± 0.9) infarctions. The extents of enhanced regions in acute infarctions were not different after intravascular (16.0 ± 1.3%) or extracellular (17.1 ± 1.7%) agents; at 8 weeks the extent of extracellular enhanced and TTC regions were smaller (13.2 ± 1.4% and 12.0 ± 1.5%, respectively). Masson’s trichrome stain demonstrated dense scar tissue, signaling the complete healing of infarction. The vascular stain showed that scar tissue contained fewer microvessels oriented in a haphazard array.

CONCLUSIONS
The combination of intravascular and extracellular Gd-chelates discriminates acute from chronic infarctions on delayed images. This double contrast agent approach can be used to determine the age and extent of infarctions. (J Am Coll Cardiol 2006;48:1961–8) © 2006 by the American College of Cardiology Foundation

Delayed contrast enhanced magnetic resonance imaging (MRI) has been shown to be highly effective for demarcating infarcted from normal myocardium (1–4). It is now clear that delayed enhancement occurs for both acute (necrosis) and chronic (scar) myocardial infarctions (MIs); as such it can be useful in various clinical circumstances. In acute cases, it is capable of distinguishing infarcted from stunned myocardium and confidently excluding MI in the setting of acute chest pain. Furthermore, in chronic cases, such as ischemic cardiomyopathy, it can be used to distinguish viable hibernating from infarcted myocardium (1).

Such discrimination allows for the prediction of recovery of dysfunctional myocardium after revascularization. A limitation of delayed contrast enhancement MRI with standard extracellular gadolinium (Gd)-chelates is its inability to distinguish acute from chronic infarctions (5,6). This limitation might have relevance in some clinical scenarios, such as acute chest pain in patients with prior infarctions or detection of intraoperative or postoperative infarction in patients with prior infarction.

It was also demonstrated that intravascular Gd-chelates enhance acute infarctions (7–9). Because the distribution volume and mechanism of distribution of intravascular and extracellular MR contrast agents differ considerably (9,10), the enhancement pattern in acute myocardial infarctions (AMIs) and chronic infarctions might also be different. This seems likely because vascular integrity is destroyed in acutely infarcted myocardium but is intact in scar tissue (11–14). The hypothesis of the current study was that intravascular Gd-chelates produce delayed contrast enhancement of acute but not chronic MIs.
Abbreviations and Acronyms

- AMI = acute myocardial infarction
- D = Dalton
- Gd = gadolinium
- IR-GRE = inversion recovery gradient echo
- LV = left ventricle/ventricular
- MI = myocardial infarction
- MRI = magnetic resonance imaging
- SI = signal intensity
- TE = echo time
- TR = repetition time
- TSE = turbo spin echo
- TTC = triphenyltetrazolium chloride

**METHODS**

**Intravascular and extracellular MR contrast media.** The intravascular contrast agent (P792, Vistarem, Guerbet Group, Paris, France) is a Gd macrocyclic compound based on the structure of extracellular MR contrast agent Gd-1,4,7,10-tetraazacyclodecane-N,N1,N11,N111-tetraacetic acid (DOTA) (Dotarem, Guerbet) supplemented by hydrophilic arms (Guerbet) (15). Preliminary studies in healthy volunteers have indicated that this agent produces no side effects (16). The chemical structure of the new intravascular contrast agent has been optimized to provide: 1) a high r1 relaxivity for MRI, 2) a high biocompatibility profile, and 3) a large molecular volume.

The relaxivities, r1 and r2, of the intravascular contrast agent were 29 and 65 l · mmol−1 · s−1, respectively, at 4% human serum albumin (HSA) at 37°C and 60 MHz (1.5 T). The r1 and r2 of the extracellular agent were 3.5 and 4.2 l · mmol−1 · s−1, respectively, at 60 MHz (1.5 T). This new agent has a molecular mass of 6,473 D, and a diameter of 5 nm, which restricts fast diffusion out of normal microvessels but is small enough to be filtered by the kidney. The extracellular agent has a molecular mass of 561 D, and a 0.9-nm diameter, which allows its distribution in the interstitium as well (17,18).

Preliminary studies were conducted in 4 pigs with a lower dose of 0.013 mmol/kg intravascular agent as recommended in previous studies (19,20). In these 4 animals, 0.013 mmol/kg produced poor enhancement of acutely infarcted myocardium. A higher dose (0.026 mmol/kg) was subsequently used in the 8 animals of this study. This concentration is one-fourth of the recommended dose (0.1 mmol/kg) of extracellular Gd-chelates on a molar Gd basis.

**Surgical procedure.** This investigation conformed to the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (Publication No. 85-23, revised 1996). Eight pigs were pre-medicated with ketamine (20 mg/kg), xylazine (2 mg/kg), and atropine (0.04 mg/kg) and anesthetized by isoflurane/oxygen (1.5% to 2.0%). After median sternotomy, the left anterior descending coronary artery was isolated at the second diagonal and occluded for 2 h, followed by reperfusion. Arterial blood pressure and heart rate were recorded during imaging via a femoral catheter.

At 3 days and 8 weeks after infarction, animals were re-anesthetized and MRI studies were acquired. Eight weeks after infarction is widely considered chronic infarction in humans (21), pigs (22), sheep (23,24), and dogs (6). Animals were then killed, and the hearts were excised and sliced (10 mm). Slices were stained with triphenyltetrazolium chloride (TTC) (2% at 37°C) to delineate scar tissue. The body weight increased from 30 to 32 kg at 3 days to 52 to 55 kg at 8 weeks.

**MRI.** A 1.5-T MR scanner (Philips Medical Systems, Best, the Netherlands) was used. A 5-element phased-array cardiac coil was wrapped around the chest. Three pulse sequences were used: 1) T2-turbo spin echo (TSE), 2) inversion recovery gradient echo (IR-GRE), and 3) T1-TSE. The T1-TSE sequence was used in this study to confirm the IR-GRE findings. The following parameters were used: echo time (TE) / repetition time (TR) = 2.1/4.4 ms for IR-GRE, TE/TR = 20 ms/1 R-R interval for T1-TSE, and TE/TR = 100 ms/2 R-R interval for T2-TSE. The inversion times used for IR-GRE sequences were optimized to null normal myocardial signal (350 to 475 ms for baseline, 275 to 400 ms for intravascular enhanced, and 250 to 350 ms for the extracellular enhanced images). A contiguous long-axis view was necessary to determine the short-axis view on IR-GRE and T1-TSE. Images encompassing the entire left ventricle (LV) were acquired at baseline and 10, 25, and 40 min after administration of the intravascular agent. After 75 min, a 0.1-mmol/kg extracellular agent was administered. Inversion recovery gradient echo and T1-TSE images were acquired after 20 min. The extent of infarctions and regional signal intensity (SI) of infarcted and normal myocardium were measured on all slices (10 mm) by 2 observers. The SI ratios were calculated by dividing SI of the infarct by remote myocardium. A threshold of +2 SD above the mean SI of remote myocardium was used to define the infarcted region (25).

**Histology.** Postmortem tissue samples were obtained from normal, interface between remote and scarred myocardium (peri-infarction zone), and scar tissue. The samples were fixed in 10% formalin and embedded in paraffin. Sections (5 μm) were stained with hematoxylin and eosin. Masson’s trichrome stain was used to define scarred myocardium. Biotinylated Bandeiria simplicifolia Isolectin B4 (Vector Laboratories, Burlingame, California) was used to specifically localize vascular endothelial cells (26).

**Statistical analysis.** The spatial extent of enhancement was expressed as a percentage of LV mass on a slice-by-slice basis. The MR sections and TTC slices were matched on the basis of anatomic landmarks, which include LV shape, papillary muscle shape, and the location and insertion point of the right ventricle. Breath hold during the acquisition of the volumetric data was used to minimize the spatial variations due to respiratory motion. Furthermore, to have
precise correlation, short-axis planes were used in MRI and histological slices. In TTC the mean of the upper and lower faces of each slice was calculated. Thus, the nesting effect played a negligible role in the measurements. The statistical differences between mean values (mean ± SEM) of SI ratios and the extent of infarctions on MR images were evaluated with the use of StatView 5.0 (SAS Institute, Cary, North Carolina). Analysis of variance was used to compare the changes in SI ratio versus time. It was also used to determine differences in the extent of infarctions on contrast enhanced images and TTC histochemical staining. This was followed by Scheffe’s F test. The null hypothesis was rejected for p < 0.05.

RESULTS

AMIs. Acute myocardial infarctions were visible on T2-TSE (SI ratio = 1.5 ± 0.1) (Fig. 1) but not on IR-GRE (1.0 ± 0.1) or T1-TSE (1.0 ± 0.1). After the administration of intravascular and extracellular agents, all acutely infarcted hearts (n = 8) showed delayed enhancement (Figs. 2 and 3). The intravascular Gd-chelate did not enhance chronic infarctions (Figs. 2 and 3). Mean changes in SI ratio versus time on contrast enhanced IR-GRE and T1-TSE sequences are shown in Figure 4. At 40 min after administration of intravascular Gd-chelate, the SI ratio was 5.6 ± 1.3 on IR-GRE and 1.5 ± 0.1 on T1-TSE sequence for acute infarctions (Fig. 5). These data indicate that IR-GRE is superior to the T1-TSE sequence. The extracellular Gd-chelate did not enhance T1-TSE images. The magnitude of signal enhancement of remote myocardium after administration of the intravascular agent was not significantly different between chronic (5.4 ± 0.31 before contrast injection and 7.8 ± 0.25 after) and acute (5.8 ± 0.46 arbitrary units before and 8.7 ± 0.61 at 5 min after injection) infarctions on T1-TSE images. A similar finding was observed on IR-GRE (1.1 ± 0.1). Of note, the extracellular Gd-chelate provided equivalent enhancement of chronic infarctions (SI ratio = 7.5 ± 0.9), as it did in acute infarctions (SI ratio = 7.7 ± 1.4), on IR-GRE (Fig. 5). Chronic infarctions did not show differential increase in SI compared with normal myocardium on T2-TSE or T1-TSE after administration of intravascular Gd-chelate (SI ratio = 1.1 ± 0.1).

Infarction extent. The extents of acute infarctions were not significantly different after administration of extracellular (17.1 ± 3.1% of LV mass) and intravascular Gd-chelates (16.0 ± 1.3%). The extents of the enhanced regions were smaller (13.2 ± 1.4% of LV mass, p < 0.01) at 8 weeks than at 3 days but comparable to true infarctions on TTC (12.0 ± 1.5% of LV mass, p = NS). The LV mass/body weight ratios (g/kg) showed no significant increase over the course of 8 weeks (3.00 ± 0.12 at 3 days and 2.92 ± 0.15 at 8 weeks). The extents of chronic infarctions were not measured on T1-TSE after administration of intravascular contrast agent or T2-TSE, because these sequences did not provide differential contrast.

Hemodynamic measurements. In acute and chronic infarctions, the heart rates were 94 ± 5 beats/min and 89 ± 3 beats/min (p = NS) and the mean arterial pressures were 71 ± 1 mm Hg and 73 ± 4 mm Hg (p = NS), respectively. Administration of intravascular and extracellular Gd-chelates caused no significant change in the mean systolic (94 ± 2 to 90 ± 3 mm Hg) or diastolic (54 ± 2 to 57 ± 3 mm Hg) blood pressures in acute and chronic infarctions. Similarly, there was no significant change in arterial oxygen saturation (96 ± 2% vs. 97 ± 3%) after contrast injections.

Histology. Hematoxylin and eosin and Mason’s trichrome stains showed no evidence of dense collagen or remodeled.

Figure 1. Long- and short-axis views T2-turbo spin echo. Magnetic resonance images acquired from 2 animals (top and bottom rows). Acute (left) but not chronic (center) infarctions showed differential enhancement (arrows). The bright signal in the left ventricular chambers (arrowheads) represents slow flowing blood adjacent to infarction. Triphenyltetrazolium chloride staining (right) shows the extent and location of infarction.
blood vessels in remote myocardium. Biotinylated Bandeiria simplicifolia isolectin B4 stain showed numerous and uniformly distributed microvessels coursing in parallel with myofibers. However, scar tissue consisted of dense collagen and remodeled, sparse, and large thick-walled blood vessels (Fig. 6). Masson’s trichrome stain showed dense fibrous tissue signaling complete healing of infarction. Biotinylated Bandeiria simplicifolia isolectin B4 stain stained vascular endothelial cells brown. This stain showed the scarcity and haphazard orientation of intact microvessels in scar tissue compared with remote myocardium.

DISCUSSION

The major findings of this study are the following: 1) intravascular Gd-chelate can discriminate AMI from scar tissue, and 2) the extents of acute infarctions delineated by intravascular Gd-chelate are the same as that shown by standard extracellular Gd-chelate. There was no delayed enhancement produced by intravascular Gd-chelate in chronic infarctions. The capability of distinguishing AMIs from scar tissue by intravascular Gd-chelate might be useful for excluding: 1) a new acute infarction in the clinical setting of known prior infarction or in the presence of ischemic cardiomyopathy, and 2) intraoperative or postoperative infarction in a patient with known prior infarction after revascularization.

There are other reported approaches for distinguishing AMIs from scar tissue (27–31). Egred et al. (27) indicated that scar tissue could be differentiated from viable myocardium with T2* blood oxygen level dependent (BOLD) MRI. However, other investigators found difficulty in assessing the infero-lateral LV walls (the areas supplied by the right and circumflex coronary arteries) because of susceptibility artifacts (28). In a recent study, Hillenbrand et al. (31) found that the combination of ^23^sodium and contrast enhanced MRI provided temporal characterization of acute infarctions and infarct healing. A possible mechanism involved in the decrease of ^23^sodium signal on MRI during infarct healing includes granulation and collagen deposition. In another study Abdel-Aty et al. (30) used a combination of triple inversion recovery T2-weighted and delayed contrast enhanced MR sequences to differentiate AMIs from scar tissue in 73 patients by showing myocardial edema in acute infarctions. Patients (n = 15) with MIs were studied on day 1 and 3 months after infarction with black-blood, T2-weighted triple inversion recovery sequence. The T2-
TSE sequence used in the current study is less sensitive to edema than the T2-weighted triple inversion recovery sequence employed in a previous study (30). This was shown by a SI ratio of 1.5 ± 0.1 on T2-TSE in the current study compared with 2.9 ± 1.5 on T2-triple inversion recovery sequence. Acute MIs showed differential contrast (high signal) on T2-weighted sequences or enhancement by extracellular and intravascular Gd-chelates. After 8 weeks the infarctions were no longer visible on T2-weighted sequences or after administration of intravascular Gd-chelate. Conversely, standard extracellular Gd-chelate produced differential enhancement between infarcted and remote myocardium in both acute and chronic infarctions. The new double contrast approach yields higher SI ratios (SI ratio 5.8 to 7.5) than T2-triple inversion recovery imaging (SI ratio 2.9) (30).

This study confirms the results of previous studies (5,6) that the magnitude of enhancement of acute and chronic infarctions is identical after administration of extracellular Gd-chelates, which eliminates the possibility of discriminating AMI from scar tissue. At the cellular level, Arheden et al. (32) and Klein et al. (31) found in acute and chronic infarctions an almost 2-fold increase in distribution volume of extracellular Gd-chelates compared with remote myocardium. Thus, delayed enhancement after administration of extracellular Gd-chelates is independent of infarction age or differences in cellular and microvascular structures. Delayed enhancement in acute infarctions can be attributed to: 1) the increase in extracellular space consequent to loss of membrane integrity (32,33), and 2) delayed wash-out kinetics of extracellular contrast media (34,35).

At the microvascular level, leakage of intravascular Gd-chelates in acute infarctions is relatively slow compared with extracellular Gd-chelates (8–10,19,20,36–38). In recent studies (19,20), investigators found that intravascular Gd-chelates need 25 to 40 min to reach peak enhancement in reperfused and occlusive infarctions, respectively, whereas the extracellular Gd-chelates require 6 min. Thus, this is the minimal time necessary to reliably exclude contrast enhancement of intravascular agents. The relatively slow transport of intravascular Gd-chelates shown in this and previous studies can be explained by: 1) microvascular obstruction (37,38), 2) high gradient pressure in the interstitium due to edema (9,37), and 3) slow convection process compared with passive diffusion transport (9,12,13,36). In a previous study (8), changes in SI on T1-weighted spin echo MRI and histopathology were correlated in acute, subacute, and chronic infarctions. Karyolysis, cytoplasmic hypereosinophilia, loss of myocytes striations, and intramyocardial hemorrhage, suggesting microvascular damage, were observed in acute infarctions. In this pathological structure, intravascular agent provided differential enhancement. In subacute infarctions, the myocytes have been replaced by reparative tissue consisting of fibroblasts and inflammatory cells. Few microvessels were observed at the peri-infarction zone. On contrast-enhanced MRI, the enhancement of subacute infarctions was less pronounced compared with acute infarctions. In chronic infarctions the myocytes had evolved to a dense fibrous scar with scarce microvessels. The intravascular agent produced no differential enhancement of scar tissue. Microscopic examinations of scar tissue in swine...
showed thick-walled large blood vessels and scarce microvessels (Fig. 6).

The absence of delayed enhancement in chronic infarctions after intravascular Gd-chelates can be attributed to the presence of residual intact and/or remodeled microvessels, which retain intravascular Gd-chelates (11,14). Hong et al. (11) observed that small intramyocardial coronary arteries undergo remodeling with an increase in wall thickness and a decrease in lumen in swine model subjected to severe myocardial ischemia. Poor vascularization and perfusion in scar tissue have also been demonstrated in scarred myocardium with fluorescent staining and microspheres, respectively (11,34). Wang et al. (13) observed a significant reduction in total vascular surface areas at 1 and 4 weeks after infarction. This decline in vascular surface area was coupled with a 75% decrease in the number of microvessels in scarred myocardium at 4 weeks after infarction.

The similar size of acute infarction demarcated by intravascular and extracellular Gd-chelates suggests that both agents are suitable for measuring the extent of acute infarction (19,20), but they differ with aging of the infarction, as this investigation has shown. The extent of infarction after administration of extracellular Gd-chelate was smaller at 8 weeks than at 3 days. Such a decline in the extent of infarction has been shown clinically (39) and experimentally (6).

**Study limitations.** A limiting factor of the current study is that infarcts of only 2 time durations (3 days and 8 weeks) were studied. Eight weeks after infarction seems reasonable, because it has been widely considered chronic infarction in humans and animals (21–24). The use of 2 MR contrast media for discriminating acute from chronic infarctions is rather time consuming, and this might limit the clinical application.

**Figure 4.** Plots show average changes in signal intensity (SI) ratio after administration of the intravascular agent in acute and chronic infarctions with inversion recovery gradient echo (top) and T1−turbo spin echo (bottom) magnetic resonance imaging. On both sequences, the intravascular agent significantly increased the SI ratio in acute but not chronic infarctions. *p < 0.01 compared with baseline SI ratio in acute infarctions; †p < 0.01 compared with SI ratio at 10 min; #p < 0.01 SI ratio of chronic compared with acute infarctions.

**Figure 5.** Histograms show the signal intensity (SI) ratios after administration of the intravascular (top) and extracellular (bottom) gadolinium-chelates. T2−turbo spin echo (TSE) and the intravascular agent provided differential enhancement of acute (black bars) but not chronic (white bars) infarctions on T1−TSE and sequences. The extracellular agent provided the same magnitude of enhancement in acute and chronic infarctions on T1−TSE and inversion recovery gradient echo (IR-GRE) sequences. Note that contrast enhanced IR-GRE sequence provides better contrast than both T1−TSE and T2−TSE sequences. *p < 0.01 SI ratios compared with acute infarctions. †p < 0.01 SI ratios compared with contrast enhanced magnetic resonance imaging (MRI).
Practical application. The current and previously reported approaches (29,31) might provide the means to age MIs. We propose that discrimination of AMIs from scar tissue can be done in the following manner: 1) in AMIs, there is delayed enhancement by both intravascular and extracellular Gd-chelates; and 2) in chronic infarctions, there is delayed enhancement by extracellular but not by intravascular Gd-chelates. We conclude that if there is no delayed enhancement after administration of intravascular Gd-chelates, it is not an acute infarction. Standard extracellular agents would also be preferable for discriminating nonviable from stunning and hibernating myocardium in cases of ischemic cardiomyopathy (1).

Reprint requests and correspondence: Dr. Maythem Saeed, Department of Radiology, University of California San Francisco, 513 Parnassus Avenue, HSW 207B, San Francisco, California 94134-0628. E-mail: Maythem.Saeed@radiology.UCSF.edu.

REFERENCES


