Imaging Time After Gd-DTPA Injection Is Critical in Using Delayed Enhancement to Determine Infarct Size Accurately With Magnetic Resonance Imaging

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Background—In patients with acute myocardial infarction (MI), delayed enhancement is seen in MRI 5 to 7 minutes after gadolinium–diethylenetriamine pentaacetic acid (Gd-DTPA) injection, and the enhancement occurs in regions that later show recovery of function. However, in a canine model of acute MI, delayed enhancement 20 to 30 minutes after injection only occurs in necrotic regions and not in surrounding, reversibly injured myocardium. The objective of the present study was to determine (1) if the size of the enhanced region varies with time after Gd-DTPA injection and (2) if and when the size of the enhanced region corresponds to the true infarct size.

Methods and Results—The left coronary artery was occluded in 15 Lewis rats for 30 minutes (n=9) or 2 hours (n=6); this was followed by reperfusion. MRI scans were performed 48±2 hours after MI. Midventricular short-axis images were obtained continuously for 40 minutes after Gd-DTPA injection (0.3 mmol/kg). The sizes of enhanced regions at each time were determined by threshold analysis and compared with triphenyltetrazolium chloride–stained sections of the excised rat heart. In all animals, the enhanced region overestimated infarct size (28±5%) immediately after the injection of Gd-DTPA, although it then gradually receded to match the size of the infarct. The time required for enhancement to accurately determine infarct size was significantly different between 2-hour infarcts (16±2 minutes) and 30-minute (26±4 minutes) infarcts (P<0.05).

Conclusions—In reperfused acute MI, accurate determination of infarct size by delayed enhancement MRI requires imaging at specific times after Gd-DTPA injection, and this time varies with the duration of occlusion. (Circulation. 2001;104:2838-2842.)

Key Words: myocardial infarction ■ magnetic resonance imaging ■ coronary disease ■ contrast media

Identification of viable myocardium is important in both chronic and acute myocardial infarction (MI). In the setting of chronic MI, the identification of multiple segments of dysfunctional yet viable myocardium indicates that revascularization procedures could help the patient’s long-term prognosis by improving left ventricular (LV) function.1,2 MRI has shown the ability to determine regional myocardial viability in patients with chronic MI by imaging regions of delayed enhancement after the injection of gadolinium–diethylenetriamine pentaacetic acid (Gd-DTPA). In chronic MI, the delayed enhancement seems to occur only in irreversibly injured tissue and, therefore, it can be used to predict which segments will experience functional recovery after revascularization and which segments will not.3–5

In the setting of acute MI, the identification of viable myocardium is important for patient management. In the first few days after MI, viability is important for risk stratification and evaluating myocardial salvage. However, the meaning of delayed enhancement in acute and subacute MI is less clear than it is in chronic MI. Studies in a canine model with 2-day-old reperfused infarcts show that regions of enhancement directly correspond to regions of infarcted tissue.6,7 Studies in humans also show that no functional recovery is seen in areas of transmural delayed enhancement in humans.3

However, other studies in humans conducted 3 to 5 days after reperfused MI have shown that some regions of enhancement recover function 3 months later.8,9 Studies in rat and canine models conducted 1 hour to 1 day after reperfused MI show that the delayed enhancement zone overestimates the infarct zone by 10% to 20%, again suggesting that enhancement occurs in both reversibly and irreversibly-injured myocardium.10,11 Together, these studies suggest that in the acute and subacute setting, enhancement may occur in both reversibly and irreversibly-injured myocardium.
There are several possible reasons that disparate results have been reported using delayed enhancement in the setting of acute MI. First, the different studies used different MR pulse sequences that could affect image quality and the degree of contrast enhancement in the image. However, the different pulse sequences should not directly affect the size of the enhanced zone or the type of tissue that enhances after Gd-DTPA injection. Second, there could be differences in the myocardium of dogs, rats, and humans that may cause enhancement in irreversibly injured myocardium in one species but not in another.

A third possible reason for the disparate results could be differences in the timing of image acquisition after the injection of Gd-DTPA. For example, the studies in humans that reported eventual recovery of function in regions that had previously been enhanced were conducted 5 to 7 minutes after Gd-DTPA injection. In the animal studies that show a direct correlation between the regions of enhancement and infarcted myocardium, imaging was performed 20 minutes after Gd-DTPA injection. The goals of this study were to (1) examine whether the size of the enhanced zone changes with the time imaging is done after the injection of Gd-DTPA in reperfused acute MI and (2) determine the time after Gd-DTPA injection at which the size of the enhanced zone accurately predicts infarct size.

**Methods**

**Animal Model**

This study conformed to the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health and was performed with the approval of the Institutional Animal Care and Use Committee at the University of Virginia.

A total of 18 Lewis rats were anesthetized with 100 mg/kg intraperitoneal sodium pentobarbital. The chest was opened with a parasternal incision, and a 6-0 silk suture was passed with a tapered needle underneath the left anterior descending coronary artery at the parasternal incision, and a 6-0 silk suture was passed with a tapered needle underneath the left anterior descending coronary artery at the level between the right ventricular outlet and the left anterior descending coronary ligation was achieved by tightening the suture over a piece of PE-200 tubing. Successful coronary occlusion and reperfusion were confirmed by significant ECG changes and color changes in the area at risk. The coronary occlusion was maintained for 2 hours in 6 animals and for 30 minutes in 12 animals. Reperfusion was achieved by removing the PE-200 tubing.

Forty-eight hours after reperfusion, the rats were sedated with 12 mg/kg intraperitoneal diazepam, and an intravenous line was implanted in the femoral vein for subsequent injection of contrast agent. Diazepam had minimal effects on blood pressure and heart rate, and the rats remained conscious during imaging.

**Preliminary MR Studies to Select Inversion Delay Time**

The introduction of Gd-DTPA into the bloodstream results in an uptake of the contrast agent into both normal and injured myocardium. The increased distribution volume and altered washout characteristics in injured myocardium result in preferential accumulation of Gd-DTPA and produce a shorter T1.11-13

To obtain optimal contrast between the normal and infarcted myocardium after Gd-DTPA injection, a series of preliminary scans were performed on 3 animals with 2-hour infarcts and 48 hours of reperfusion. These animals were not part of a study group, but they had identical surgeries, occlusion times, and reperfusion times as the animals in the study groups. MRI scans were performed in a 1.5 Tesla Siemens Magnetom Vision using a quadrature array wrist coil (Medical Advances). Image acquisition was gated to the ECG signal using an external ECG monitoring system (In Vivo Research). Animals were placed prone within the wrist coil. A midventricular short-axis slice 6 mm from the apex of the LV was selected for study.

An ECG-gated, spin-echo sequence with a nonselective inversion prepulse was used with a repetition time of 3 to 4 beats (900 to 1200 ms), an echo time of 20 ms, a field of view of 90 mm, a rectangular field of view of 3/4, and a matrix size of 192x256. A spin-echo sequence was used because it has an inherently greater signal-to-noise ratio than a gradient echo sequence, which allowed us to use finer image resolution. The segmented gradient echo sequence is more commonly used for viability imaging because it can be acquired more rapidly, but for this application we chose the increased signal-to-noise ratio and compromised on acquisition speed. A trigger delay time based on the heart rate of the individual rat was added to acquire images at end-diastole.

The pulse sequence was used with inversion delay times varying from 100 to 400 ms in 50-ms steps. Signal intensities within the normal and infarcted tissue on the images at each inversion time were interrogated to yield an inversion recovery curve for each tissue and to select an optimal inversion time.

**Time-Course Study of Gd-DTPA Enhancement**

To study the time course of enhancement, the animal preparation and MR imaging was identical to the procedures described above in the preliminary studies. Animals were sedated with intraperitoneal diazepam 48±2 hours after reperfused MI. A short-axis slice was selected with the center of the slice 6 mm from the apex of the LV to obtain a position that could be accurately correlated with histological samples. Cine images were obtained at this location to verify dysfunction in the LV wall. Parameters for cine imaging were as follows: field-of-view, 90 mm; matrix, 192x256; slice thickness, 3.0 mm; echo time, 15 ms; and 6 time points over the cardiac cycle. Gd-DTPA (0.03 mmol/kg) was then injected over 30 s through the indwelling line previously placed in the femoral vein. Imaging was started immediately after the completion of the 30-s injection using the inversion-recovery–prepared spin-echo sequence described in the preliminary study. Images were acquired continuously for 40 min-
utes at the same short-axis location where cine imaging had been performed earlier. Each image was acquired over a period of 2 to 3 minutes (depending on heart rate), which determined the temporal resolution of the time-course study. A single slice was acquired to maximize the number of time points acquired after injection.

The MR images were imported into the software package Matlab (MathWorks, Inc) for analysis. Endocardial and epicardial boundarys were manually traced in the cine images. The boundaries from the mid-diastolic time point (corresponding to the time point when the time-course images were acquired) were used as templates to determine myoccardial boundaries on the time-course images. The size of the enhanced zone on the time-course images was determined by threshold analysis. A region was considered “enhanced” if the signal intensity was 2 SDs above the signal intensity in a remote region of normal myocardium. The size of the short-axis myocardial slice and the size of the enhanced zone were determined at each time point for all animals. An experimental time line is shown in Figure 1.

**Tissue Analysis**

The rats were euthanized and hearts were excised immediately after collecting MR images. Hearts were cross-sectioned into 3-mm-thick short-axis slices for alignment with the MR images. The short-axis slices of myocardium were stained with triphenyltetrazolium chloride (TTC) to determine true infarct size. Digital photographs were taken from both sides of the LV cross-sections located 6 mm from the apex for comparison with the corresponding MR images. On both sides of the selected LV cross-section, the endocardial and epicardial borders of the entire LV and the pale infarcted regions were traced by a physician trained in cardiac pathology (Z.Y.). For each side of each slice, the pixel count obtained from the infarcted region was divided by the pixel count obtained from the entire LV cross-section. Infarct and LV sizes were then taken as the average obtained from the 2 sides of each short-axis cross-section.
Statistical Analysis

Differences between the size of the enhanced zone and the true infarct size determined by TTC stain were calculated as a percent of LV area for each animal at all time points after injection. Data from the animals with 30-minute infarcts and those with 2-hour infarcts were compared with a 2-sided t test, assuming unequal variances and unequal sample sizes (Behrens-Fisher problem).14

Results

Selection of Inversion Delay Time

The “null” point for normal myocardium after Gd-DTPA injection was 167±21 ms. The point with the maximum signal difference between infarcted and normal myocardium was 289±24 ms. There was a wide band or inversion times (200 to 400 ms) where signal difference was at least 90% of maximum. Therefore, an inversion time of 225 ms was chosen as a compromise between complete nulling of normal myocardium and maximum signal difference. This inversion time also produced consistent gating at the high heart rates present in the animals (300 to 350 beats/min).

Imaging Protocol and Exclusions

A total of 15 of the 18 animals completed the imaging protocol shown in Figure 1 (n=9 with 30-minute occlusion and n=6 with 2-hour occlusion). Three animals (all with 30-minute occlusions) could not be imaged due to poor ECG gating.

Time-Course Study

In all animals, a significant region of enhancement was seen in the anterior and lateral walls corresponding to the area supplied by the artery that was occluded and reperfused. The size of the enhanced region decreased with time after Gd-DTPA injection. The images in Figure 2 show serial time points taken from a typical time course study in one of the animals. The images begin with the first image acquired immediately after Gd-DTPA injection and continue until 30 minutes after injection. The region of enhancement gradually shrank over time to closely match the size and shape of the region of infarction defined by TTC in the post mortem tissue analysis. The initial overestimation of infarct size, followed by a gradual shrinkage to approximate the true infarct size, was typical in all animals.

Figure 3 shows selected images from a second animal. Two images after injection are shown (6 and 30 minutes after injection), as is the corresponding TTC-stained section. The enhanced zone overestimates the infarct size 4 minutes after injection (by 40% in this animal), but accurately depicts infarct size at 30 minutes after injection.

In the animal illustrated in Figure 2, a nonenhanced region within the enhanced myocardium exists at 3 minutes after injection. It represents either first-pass effects or a no-reflow zone consisting of microvascular obstruction.15 This area becomes enhanced by 9 minutes after injection. At 27 minutes after injection, the enhanced region seems to correspond to the area seen to be nonenhanced immediately after injection. A nonenhanced core region surrounded by an area of enhancement was seen in 13 of the 15 animals (7 of 9 with 30-minute infarcts and 6 of 6 with 2-hour infarcts).

Differences between infarct size, as determined by MRI and TTC, were calculated as a percentage of the LV area at all time points after injection (zero difference indicates exact area correspondence between infarct size). The results for all the animals (enhanced zone minus infarct size) are plotted in Figure 4. In both the 30-minute and 2-hour occlusion groups, infarct size was overestimated immediately after Gd-DTPA injection; however, the time required for the enhanced zone to determine infarct size correctly varied between the 2 groups. The difference between the groups was significant at 13 of 21 time points measured after Gd-DTPA injection (P<0.05 using a 2-sided, nonpaired t test).

A “time-to-correspondence” was defined as the time required after contrast injection for the area of the contrast-
Enhanced zone to correspond to the area of the infarct on the TTC images. In the 30-minute infarcts, time-to-correspondence was 26±4 minutes, and in the 2-hour infarcts, the time was 15±2 minutes. The difference was significant (P<0.05 using a 2-sided, nonpaired t test). The mean time-to-correspondence over all the animals was 21±4 minutes. Infarct size in the animals with 2-hour occlusions was 33±11% of the LV, and the infarct size in the animals with 30-minute occlusions was 24±9% of the LV.

Signal Intensity Measurements
Signal intensity in the enhanced region varied with time after injection. Immediately after the injection of Gd-DTPA, average signal intensity in the enhanced zone was elevated 610±40% above that in the remote zone. Signal intensity stayed fairly constant (no significant change, P<0.05) at this level until 18 minutes after injection. Thereafter, signal intensity began to drop slowly, such that by 30 minutes after injection, signal intensity in the enhanced zone had declined to 305±20% above that in the remote zone.

Discussion
This study demonstrates that the size of the enhanced region varies with the time imaging is performed after Gd-DTPA injection in a model of reperfused acute MI. Immediately after Gd-DTPA injection, the enhanced region overestimates the true infarct size by 20% to 40%. The time for the enhanced zone to correspond to the true infarct size was 21±4 minutes.

This study may shed some light on the disparate results reported in the literature regarding delayed enhancement in the setting of acute MI. Studies in humans with acute MI have indicated that delayed enhancement occurs in regions that later recover contractile function, suggesting that the delayed enhancement occurs in reversibly-injured myocardial tissue. Several studies in animals with acute MI have shown that the size of the delayed enhancement zone directly corresponds to the TTC-determined infarct size, suggesting that delayed enhancement occurs only in reversibly-injured (infarcted) myocardial tissue. Close inspection of the methodology of these studies reveals that the human studies were conducted ≈7 minutes after contrast injection, and the animal studies were conducted ≈20 to 30 minutes after injection. Examining Figure 4, one can see that the imaging time after injection could reconcile these 2 apparently disparate results. At 7 minutes after injection, infarct size was overestimated by 25%. At 25 minutes after injection, delayed enhancement correctly identified the infarct size. Further studies are needed to determine whether this effect also occurs in humans or other animal species, but this study does suggest that the time interval between Gd-DTPA injection and image acquisition may be critical in using delayed enhancement to estimate infarct size noninvasively.

The idea that Gd-DTPA would overestimate infarct size early after injection has been suggested previously. A possible reason for the overestimation is that early after injection, the Gd-DTPA accumulates in the peri-infarct zone of reversibly-injured myocardial tissue. This reversibly-injured tissue would be expected to be edematous and, hence, have an increased interstitial volume. The increased interstitial volume in the peri-infarct zone would increase the distribution volume for Gd-DTPA and, thus, increase the signal intensity in the reversibly-injured tissue. Other reasons for the overestimation of infarct size early after the injection of Gd-DTPA might involve altered washout characteristics of the reversibly-injured myocardium or changes in blood flow associated with ischemia/reperfusion injury.

This study also found that there was a significant difference between the time-to-correspondence for 30-minute infarcts versus 2-hour infarcts. In 30-minute infarcts, the time for the size of the delayed enhancement zone to match the true size of the MI was significantly longer than it was in the 2-hour infarcts. This might be related to the fact that infarct size is larger in 2-hour infarcts than it is in 30-minute infarcts. The size of a 2-hour MI in rodents is essentially the same as that of a permanent occlusion. It follows that the border zone of salvageable myocardium surrounding a 30-minute infarction is much larger than that surrounding a 2-hour infarction. We hypothesize that early after MI, severely-injured (but nevertheless salvaged and viable) myocardial tissue adjacent to the infarct zone has a distribution volume for Gd-DTPA that is intermediate between that of nonischemic and infarcted (ie, necrotic and/or apoptotic) myocardial tissue. Not only would this intermediate level of Gd-DTPA explain why the time-to-correspondence for 30-minute infarcts was significantly longer than for 2-hour infarcts, it might also explain the discrepancies between various clinical studies (where the extent of myocardial salvage is highly variable and exceedingly difficult to measure).

Despite the results of the current study suggesting that the time interval between the injection of Gd-DTPA and image acquisition may be important, there remain several unanswered questions regarding the use of delayed enhancement.
for the noninvasive estimation of myocardial infarct size. First, there could be a difference in how myocardial tissue in dogs, rats, and humans respond to ischemia/reperfusion injury. In particular, the distribution volume for Gd-DTPA in reversibly-injured tissue may vary between species. Studies have shown that dogs, rats, and humans have different levels of collateral development in response to chronic ischemia. Similarly, the nature of the edematous response to ischemia/reperfusion injury may vary between species. Second, there are significant differences in the MR pulse sequences used in various studies (including the current study). We used an inversion-recovery spin-echo sequence to maximize spatial resolution, whereas other groups have used a segmented, inversion-recovery, gradient-echo sequence to maximize acquisition speed and contrast. Despite using a spin-echo sequence, we were able to obtain signal intensity values comparable to those reported in other studies using gradient-echo pulse sequences.

A limitation to the current study was that the use of a whole-body scanner to image small animals requires certain compromises to achieve adequate resolution. We used a conventional spin-echo sequence, acquiring one k-space line every third heart beat, which yielded ≈4 pixels across the rat myocardium in the short-axis view; this is comparable to the resolution seen in human studies. The drawback to this sequence is that ≈600 heart cycles were required for a single slice. With the rapid heart rate of rats, the scan was <3 minutes in length, but interscan temporal resolution and multislice capability were sacrificed.

In conclusion, we found that the size of the delayed enhancement region varies with the time imaging is performed after Gd-DTPA injection. In reperfused MI, it is important to wait to ensure accurate determination of infarct size by contrast enhancement, which varies with occlusion time.

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