Magnitude and Time Course of Microvascular Obstruction and Tissue Injury After Acute Myocardial Infarction

Carlos E. Rochitte, MD; João A.C. Lima, MD; David A. Bluemke, MD, PhD; Scott B. Reeder, PhD; Elliot R. McVeigh, PhD; Toshiya Furuta, MD; Lewis C. Becker, MD; Jacques A. Melin, MD

Background—Microvascular obstruction within an area of myocardial infarction indicates worse functional recovery and a higher risk of postinfarction complications. After prolonged coronary occlusion, contrast-enhanced MRI identifies myocardial infarction as a hyperenhanced region containing a hypoenhanced core. Because the time course of microvascular obstruction after infarction/reperfusion is unknown, we examined whether microvascular obstruction reaches its full extent shortly after reperfusion or shows significant progression over the following 2 days.

Methods and Results—Seven dogs underwent 90-minute balloon occlusion of the left anterior descending coronary artery (LAD) followed by reflow. Gadolinium-DTPA–enhanced MRI performed at 2, 6, and 48 hours after reperfusion was compared with radioactive microsphere blood flow (MBF) measurements and myocardial staining to define microvascular obstruction (thioflavin S) and infarct size (triphenyltetrazolium chloride, TTC). The MRI hypoenhanced region increased 3-fold during 48 hours after reperfusion (3.2 ± 1.8%, 6.7 ± 4.4%, and 9.9 ± 3.2% of left ventricular mass at 2, 6, and 48 hours, respectively, P < 0.03) and correlated well with microvascular obstruction (MBF < 50% of remote region, r 2 = 0.99 and thioflavin S, r 2 = 0.93). MRI hyperenhancement also increased (21.7 ± 4.0%, 24.3 ± 4.6%, and 28.8 ± 5.1% at 2, 6, and 48 hours, P < 0.006) and correlated well with infarct size by TTC (r 2 = 0.92). The microvascular obstruction/infarct size ratio increased from 13.0 ± 4.8% to 22.6 ± 8.9% and to 30.4 ± 4.2% over 48 hours (P = 0.024).

Conclusions—The extent of microvascular obstruction and the infarct size increase significantly over the first 48 hours after myocardial infarction. These results are consistent with progressive microvascular and myocardial injury well beyond coronary occlusion and reflow. (Circulation. 1998;98:1006-1014.)

Key Words: magnetic resonance imaging ▪ myocardial infarction ▪ microcirculation ▪ reperfusion ▪ perfusion

Early reperfusion by thrombolytic therapy or angioplasty is now widely used to limit infarct size, preserve left ventricular (LV) function, and improve survival in patients with acute myocardial infarction. Although restoration of blood flow to previously ischemic tissue does occur after reperfusion, the process is not homogeneous, and limited myocardial perfusion is observed in some parts of the injured territory. This so-called “no-reflow” or “low-reflow” phenomenon has been documented at the inner portion of the LV wall, which often remains nonreperfused after release of prolonged coronary occlusion. Electron microscopic studies of tissue within the no-reflow region reveal severe microvascular damage and obstruction by red and white blood cells and other necrotic debris. Microvascular obstruction at the infarct core has been demonstrated in humans and represents a predictor of poor myocardial functional recovery and postinfarction cardiovascular complications.

Initially, microvascular obstruction was believed to be completed at the onset of arterial reflow, but more recent data suggest that this process is dynamic and develops up to 3.5 hours after reperfusion. However, the time course and magnitude of microvascular damage beyond reperfusion remains controversial, in large part because noninvasive methods to study this phenomenon serially were unavailable. It was recently demonstrated that microvascular obstruction can be evaluated noninvasively by contrast-enhanced MRI. In addition, this method has been shown to index infarct size by probing water kinetics through shortened proton relaxation times induced by gadolinium atoms. Numerous previous studies have established the validity of this approach to assess myocardial damage after experimental and human coronary artery occlusion. Therefore, our principal objective was to analyze the time course and magnitude of the no-reflow region using contrast-enhanced MRI up to 48 hours after coronary reflow. In addition, we aimed to validate the serial assessment of microvascular obstruction by MRI against radioactive microsphere blood flow (MBF) measurements, the established basic methodology of studying microvascular damage after myocardial ischemia.

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From the Cardiology Division, Department of Medicine (C.E.R., J.A.C.L., T.F., L.C.B.); Department of Radiology (J.A.C.L., D.A.B., E.R.M.); and Department of Biomedical Engineering (E.R.M., S.B.R.), Johns Hopkins University, Baltimore, Md, and the Division of Cardiology, Department of Medicine, University of Louvain, Brussels, Belgium (J.A.M.).
Correspondence to João A.C. Lima, MD, Johns Hopkins Hospital, Cardiology Division, Blalock 569, 600 N Wolfe St, Baltimore, MD 21287-6568.
E-mail jlima@welchlink.welch.jhu.edu
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Methods

Experimental Protocol

Seven mongrel dogs (mean weight, 22±2.3 kg) were initially anesthetized with thiopental (26 mg/kg IV), intubated, and mechanically ventilated. They received halothane anesthesia during catheterization procedures and small intravenous (IV) boluses of sodium pentobarbital during MRI (2.4 to 4.9 mg/kg; the mean total dose was 20.3 mg/kg for the first day of the protocol).

Through a right femoral artery catheter sheath, a 7F pigtail catheter was placed into the LV cavity and used for microsphere administration and blood pressure monitoring. Microsphere reference blood samples were obtained from the femoral artery catheter sheath. An IV bolus of heparin (3000 IU) was given before the left coronary artery was accessed with a JR 4 catheter introduced through a right carotid arterial sheath. Baseline coronary angiography was performed to prove left anterior descending coronary artery (LAD) patency. Then an angioplasty balloon of 3-mm diameter was inflated (at 4 atm) to occlude the LAD. Angiography was repeated to document coronary occlusion. Reperfusion was established after 90 minutes of total LAD occlusion.

After balloon deflation, LAD patency was documented by left coronary angiography. The animals were allowed to recover from anesthesia and were kept alive during the next day. On the third day, the animals were anesthetized with repeated IV boluses of sodium pentobarbital (2.4 to 4.9 mg/kg; mean total dose, 41.3 mg/kg) and also received an IV bolus of heparin (3000 IU) before undergoing repeat coronary angiography to document LAD patency 48 hours after experimental infarction.

At the end of the experimental protocol, the anesthetized animals received 20 mL of thioflavin S 4% solution through an LV catheter to define the region of microvascular obstruction (no-reflow region), as previously documented.4,8

MRI Protocol

Contrast-enhanced MRI studies were performed at 2, 6, and 48 hours after reperfusion. T1-weighted images were obtained in a 1.5-T system. The animals were placed in the left lateral decubitus position with a flexible radiofrequency coil wrapped around the chest. We used a fast gradient-echo imaging pulse sequence, spoiled gradient recalled (SPGR) acquisition in the steady state, described in detail elsewhere.35 This pulse sequence included nonselective preparatory radiofrequency pulses used to drive magnetization to a steady state before image acquisition, which resulted in homogeneous and dark precontrast baseline images. With these parameters, pixel intensity approximates a linear relation to 1/T1, which is linearly related to changes in contrast concentration over a wide range of pixel intensities.10 The imaging parameters were matrix, 256×108; flip angle, α=45°; field of view, 32 cm; TR=6.5 ms; TE=2.3 ms; slice thickness, 10 mm; and voxel dimensions, 1.2×3.3×10 mm. Images were obtained at ECG-gated diastolic phase (delay after R wave on ECG, 180 to 250 ms) and during mechanical ventilation pause at the same time point in the respiratory cycle.

The contrast-enhanced imaging protocol began 10 seconds after a 0.225-mmol/kg bolus injection of Magnevist (gadopentetate dimeglumine, Berlex) in the femoral vein and continued for 15 minutes thereafter. During the first 5 minutes of postcontrast injection, y resolution was 108 lines, and one third of k-segmented space was acquired during each cardiac cycle (36 phase encodes per heartbeat). With 4 to 5 short-axis myocardial slices imaged 4 times during each breath-hold. Late images (10 to 15 minutes after contrast injection) were acquired with greater spatial resolution in the y axis (252 lines) but with short-axis slices imaged only twice during each breath-hold. Breath-hold duration was kept constant (20 to 30 seconds) throughout the entire imaging protocol. Between breath-holds, animals were ventilated for at least 30 seconds.

MRI Data Analysis

With the NIH Image software tool (developed by the US National Institutes of Health, Bethesda, Md) on a Macintosh computer, the LV boundaries and regions of myocardial hypoenhancement and hyperenhancement were delineated on contrast-enhanced images by 2 observers blinded to the postmortem data. The endocardial and epicardial contours were defined on the early images (first 3 minutes after contrast injection). At this time, the high concentration of contrast present in the LV cavity provides ideal image contrast against the low myocardial contrast penetration, allowing an accurate delineation of the endocardial and epicardial contours. These contours were then pasted onto the late images (10 to 15 minutes after contrast injection, Figure 1).

Three patterns of myocardial signal enhancement were identified by sequential analysis of all images obtained during the 15 minutes of image acquisition as previously described.4,8 Briefly, in noninfarcted myocardium, signal intensity rose rapidly in the first minute after contrast bolus injection and then decayed progressively over the next 10 to 15 minutes. In infarcted regions, a similar sharp rise in myocardial signal intensity in the first postcontrast minute was followed by a continued rise in signal intensity over the following 2 to 3 minutes and then by a much slower decay, leading to myocardial hyperenhancement relative to normal noninfarcted regions in images obtained 10 to 15 minutes after contrast bolus injection (Figure 1). The third pattern was characterized as a slow rate of signal intensity increase in the first 3 minutes, which followed contrast administration. This pattern of myocardial hyperenhancement relative to the surrounding myocardial regions characterizes the region of microvascular obstruction (no-reflow region), as previously documented.4,8
The hypoenhanced regions were defined as those regions that showed distinct and persistent hypoenhancement for at least 1 minute during the first 3 minutes after contrast injection (early images). From a series of early images (acquired at 8-second intervals), for each short-axis slice, hypoenhanced regions were selected from the image containing the greatest area of myocardial hypoenhancement. The hypoenhanced regions were defined as distinct myocardial brightness on late images (10 to 15 minutes after contrast injection), and its extent was measured by planimetry on the image showing the largest bright region.

The extents of infarcted and microvascular-obstruction regions defined by MRI as a percentage of the total LV mass was calculated as the sum of the regions of interest for all slices divided by the sum of the LV cross-sectional areas from all slices, as previously described:\%

\begin{equation}
\text{hypoenhancement area of all slices/} \Sigma \text{LV cross-sectional area of all slices}.
\end{equation}

**Postmortem Delineation of Microvascular-Obstruction, Infarcted, and Risk Regions**

As described above, the LV was sectioned into cross-sectional myocardial slices immediately after intraventricular injection of thioflavin S and cardiac arrest. Thioflavin S is a fluorescent dye first used to demonstrate the distribution and patency of the microvasculature by means of endothelial staining.\(^{11}\) It stains the endothelium of blood vessels that have received arterial flow between the time of injection and the excision of the heart, thereby defining the distribution of myocardial perfusion. Myocardial regions in which the microvasculature is stained by thioflavin will fluoresce brightly when viewed under ultraviolet light, thus delineating no-reflow regions as thioflavin S-negative regions.\(^{5}\) The apical and basal views of each myocardial slice were first drawn onto an acetate sheet and photographed under ultraviolet light (delineating regions that failed to stain by thioflavin as thioflavin-negative or no-reflow regions). Immediately thereafter, the slices were submerged into a 1\% solution of triphenyltetrazolium chloride (TTC) for 20 minutes at 37°C and again outlined on an acetate sheet and photographed under room light (delineating regions that failed to stain with TTC as TTC-negative or infarcted regions). By this protocol, viable myocardium reduces tetrazolium to formazan pigments by diaphoresis, which uses NADH or NADPH as an electron donor. Infarcted regions are identified as tetrazolium-staining defects due to loss of cofactors in necrotic myocardium.\(^{12}\)

After TTC staining, myocardial slices were sectioned into radial segments for MBF measurements. Each myocardial segment was divided into 5 equal transmural pieces: 2 subendocardial, 2 subepicardial, and 1 midwall piece. Pieces and reference blood samples were weighted and counted in a \(\gamma\)-emission spectrometer (Packard). The radioactive counting and flow calculations were performed by standard methods.\(^{13,14}\) Segments 6 to 11 mm wide were obtained inside the radioactive counting and flow calculations were performed by standard methods.\(^{13,14}\) Segments 6 to 11 mm wide were obtained inside the TTC-negative and remote regions. In addition, 5 strips 1 mm wide were excised outside each lateral border of the TTC-negative region to increase the spatial resolution of MBF measurements.

Risk regions were defined by MBF measurements as previously described.\(^{5,15-17}\) This technique has been validated in our laboratory against staining methods such as monastral blue dye injection.\(^{18}\) In brief, myocardial pieces with MBF <50\% of the MBF in the equivalent remote-region piece during coronary occlusion constituted the risk region. The precise location and size of each myocardial piece was recorded on acetate sheets, constituting the “blood-flow map.” This blood-flow map was also compared with photographs and transparencies, registering the extent and location of the infarcted and no-reflow regions defined by myocardial staining, to delineate the at-risk but noninfarcted region (the TTC-positive region with MBF <50\% at the time of coronary occlusion). Similarly, regions of microvascular obstruction defined by MBF measurements were those containing pieces with MBF <50\% relative to its corresponding remote piece at 2, 6, and 48 hours after coronary reflow, as previously described.\(^{4}\) By use of the blood-flow map, the risk and no-reflow regions were measured by planimetry (Sigma-Scan, Jandel Scientific). Very importantly, drawings were double-checked by comparison with the photographs to ensure the accuracy of all topographic measurements.

Four regions were defined on the basis of the postmortem data for blood-flow measurement purposes: the remote region, defined as the LV wall opposite the infarct; the TTC-positive/risk region, defined as the TTC-positive regions with MBF <50\% relative to remote region during coronary occlusion; the thioflavin-positive/infarcted region, defined as the TTC-negative but thioflavin-positive regions; and the region of microvascular obstruction (no-reflow region), defined as the thioflavin-negative regions.

To compare MRI-defined regions of myocardial infarction and microvascular obstruction with corresponding regions defined at postmortem examination, 3 topographic regions were defined: the region of microvascular obstruction (no-reflow region), defined by staining as the thioflavin-negative regions and by MBF measurements as regions with MBF <50\% relative to the remote region after reperfusion; the infarcted region, defined as the entire TTC-negative regions; and the risk region, defined as the regions with MBF <50\% relative to the remote region during coronary occlusion.

To calculate infarct size in terms of percent total LV mass, we took the union of the infarcted regions drawn from the apical and basal views of each myocardial slice. The total LV mass was calculated by use of the epicardial contour from the basal view and the endocardial contour from the apical view of each myocardial slice. Thus, infarct size was calculated as \% infarcted regions = \(\Sigma\) TTC-negative area of all slices/\(\Sigma\) LV cross-sectional area of all slices. The extent of risk and microvascular-obstruction regions in terms of percent total LV mass were calculated by a similar methodology.

Cross-registration of MRI with histopathological data was used in this study for qualitative comparisons only, with the sole objective of examining the equivalence of spatial localization of infarcted and no-reflow regions obtained by MRI against histopathological staining methods performed during postmortem examination. To match the locations of anatomic myocardial slices relative to MRI short-axis images, we selected the most apical short-axis image that still showed residual LV cavity. Starting at this slice location, we obtained several (typically 5) parallel, 10-mm-thick short-axis slices up to the LV base defined by the mitral valve ring. Later, on postmortem examination, we cut our first apical short-axis slice so that it contained the residual apical portion of the LV cavity, similar to that obtained by MRI. We then proceeded to section the left ventricle from apex to base in slices 1 cm thick, comparable to the image planes obtained by MRI. We then used natural LV landmarks, such as the papillary muscles and the connections between right and left ventricles, to superimpose the transparencies generated during postmortem examination onto MRI images.

**Statistical Analysis**

The extent of the regions based on MBF measurements, MRI, and myocardial staining are expressed as mean±SEM. We used repeated-measures ANOVA and the Bonferroni test for multiple comparisons of MBF measurements across time and between different regions. Simple linear regression was used to assess the correlation between MRI and postmortem or microsphere measurements. The treatment for extreme values in the analysis across time was 2-fold: a sensitivity analysis\(^{18}\) with removal of these values and a nonparametric method (the Friedman test)\(^{20}\) not influenced by extreme values.

**Results**

**Hemodynamics**

Heart rate, systolic and mean blood pressures, and the heart rate x systolic blood pressure (double product) are shown at different times during the experimental protocol (Table). There was a slight decrease in blood pressure during occlusion, with recovery 2 hours after reperfusion. Dobutamine stimulation, used primarily to assess coronary flow reserve in myocardium injured by different degrees of ischemia, caused an increase in heart rate (10.1±5%), mean blood pressure (18.0±5%), systolic blood pressure (18.0±6%), and the double product (30.8±6%, Table).
Myocardial Blood Flow

Absolute radioactive MBF measurements at different time points during the experimental protocol are shown in Figure 2. During total coronary occlusion, we found significantly lower MBFs in the thioflavin-negative region (0.05 ± 0.01 mL min⁻¹ g⁻¹), the TTC-negative/thioflavin-positive region (0.09 ± 0.01 mL min⁻¹ g⁻¹), and the TTC-positive/risk region (0.34 ± 0.03 mL min⁻¹ g⁻¹) compared with noninfarcted remote regions (0.96 ± 0.06 mL min⁻¹ g⁻¹). Two hours after reperfusion, MBF was restored in all previously underperfused regions, including the thioflavin-negative region (1.31 ± 0.39 mL min⁻¹ g⁻¹, 107.6 ± 29.0% relative to remote region). However, MBF decreased significantly in the thioflavin-negative regions 6 hours after reperfusion (0.70 ± 0.16 mL min⁻¹ g⁻¹, 45.4 ± 12.8% of remote flow) and remained at low levels up to 48 hours after infarction/reperfusion (0.55 ± 0.18 mL min⁻¹ g⁻¹, 39.5 ± 8.3% of remote flow; P = 0.005). Forty-eight hours after infarction, there were no statistically significant differences in MBF among regions outside the thioflavin-negative region. Moreover, remote noninfarcted regions showed little variation in absolute MBF during the entire experimental protocol except during catecholamine stimulation. This pattern of MBF alterations demonstrates the development of microvascular obstruction at the infarct core.

During dobutamine stimulation, there was an increase in absolute flow in all regions. However, such augmentation was progressively lower from the remote to the thioflavin-negative regions, for which changes in MBF were not statistically significant. Mean MBF increase was 85.2% in the remote, 84.4% in the TTC-positive/risk, 57.6% in the TTC-negative/thioflavin-positive (P < 0.05 by repeated-measures ANOVA with Bonferroni correction), and 25.6% in the thioflavin-negative region (ANOVA, P = NS).

Time Course of Microvascular Obstruction After Myocardial Infarction and Reperfusion

The extent of microvascular obstruction by MRI expressed as percent hypoenhanced LV mass after contrast injection was measured at 2, 6, and 48 hours after coronary reflow. The extent of microvascular obstruction by MRI at 48 hours correlated well with microvascular obstruction measured as the extent of the thioflavin-negative LV mass at postmortem examination (Figures 3 and 4, A and B). Moreover, there was also a good correlation between microvascular obstruction by MRI and by MBF measured 48 hours after reperfusion (Figure 4A and 4C).

Microvascular obstruction assessed as hypoenhanced regions on contrast-enhanced MRI increased in size in the first 48 hours after reperfusion (Figures 5 and 6). At 2 hours after reperfusion, the extent of microvascular obstruction by MRI was 3.2 ± 1.8% of the LV mass; at 6 hours, it increased to 6.7 ± 4.4%; and at 48 hours, it reached 9.9 ± 3.2% (P = 0.03 by repeated-measures ANOVA). Microvascular obstruction defined by MBF also had a progressive and statistically significant increase up to 48 hours after reperfusion, from 4.8 ± 3.3% at 2 hours to 9.7 ± 4.5% at 6 hours and to 12.5 ± 4.0% at 48 hours, with P = 0.003 (Figure 6). The extent of MRI-defined microvascular obstruction, relative to total myocardial infarct size defined as the total hyperenhanced myocardial mass, increased progressively up to 48 hours after coronary occlusion and reflow, from 13.0 ± 4.8% at 2 hours to 22.6 ± 8.9% at 6 hours and to 30.4 ± 4.2% at 48 hours (P = 0.024, Figure 7). The ratio of microvascular obstruction by radioactive microsphere to the MRI-defined infarcted region increased similarly over the same time period (P = 0.02, Figure 7B).

Because 1 of our experiments showed much greater microvascular obstruction magnitudes than the other cases at all 3 time points, measured either by MRI or by MBF measurements (Figure 4A), we used 2 additional methods to further assess the statistical significance of the alterations in microvascular damage over time. A sensitivity analysis excluding the observed extreme values provided even stronger evidence that microvascular obstruction by both MRI and MBF increases over time up to 48 hours after coronary reflow (P < 0.01 for both). In addition, a nonparametric method not influenced by extreme values, the Friedman test, was performed and also confirmed the statistical

**Table 1.** Hemodynamics

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Data are expressed as mean±SEM.
significance of the microvascular obstruction progression over time measured by both MRI and MBF ($P<0.05$ and $P<0.01$, respectively).

**Time Course of Myocardial Infarction After Coronary Occlusion and Reflow**

The extent of myocardial infarction defined by MRI correlated well with infarct size measured by TTC on postmortem examination (Figures 8 and 9). The extent of myocardial damage by MRI (28.8±5.1%) overestimated by 9.4% the TTC-negative area, which was 26.4±6.9%. Moreover, the extent of myocardial infarction was augmented 36.5±10.5% in the 2 days after coronary occlusion and reflow. Infarct extent by MRI was 21.7±4.0% at 2 hours, 24.3±4.6% at 6 hours, and 28.8±5.1% at 48 hours ($P<0.006$ by repeated-measures ANOVA, Figure 10).

The volume of LV myocardial tissue underperfused at the time of coronary occlusion (territory at risk) represented 37.2±6.0% of the total LV mass. However, because the total mass of hyperenhanced myocardium increased over time, infarct size relative to the risk region increased from 58.2±6.0% at 2 hours to 63.6±2.7% at 6 hours and 77.7±5.1% at 48 hours ($P=0.004$). In addition, 65.7±7.3% of the risk region was TTC-negative at postmortem examination, which was similar to the MRI infarct size/risk ratio at 48 hours (77.7±5.1%, $P=NS$). Finally, the size of the risk region was proportional to the extent of myocardial infarction by MRI at 2 ($y=0.62x−1.5$, $r=0.93$), 6 ($y=0.76x−3.96$, $r=0.98$), and 48 ($y=0.78x−0.32$, $r=0.93$) hours after reperfusion. A similar relationship was found between the size of the risk region at the time of coronary occlusion and TTC-negative infarct size measured at postmortem examination 48 hours later ($y=1.08x−13.71$, $r=0.95$).

**Discussion**

**Microvascular Obstruction**

This is the first study designed to investigate the time course of microvascular obstruction in the 2 days that follow coronary occlusion and reperfusion. Our results demonstrate a consistent increase in the region of microvascular obstruction defined by both radioactive microspheres and contrast-enhanced MRI up to 48 hours after acute myocardial infarction. This increase occurs over and above infarct size hypoenhancement; and Thioflavin −, thioflavin-negative regions. % LV indicates percent of total LV mass. $P$ value by repeated-measures ANOVA. B, Relationship between MRI hypoenhancement at 48 hours after reperfusion and thioflavin-negative regions expressed as % LV ($y=0.5x+3.2$, $r=0.93$). C, Relationship between extent of MRI hypoenhancement at 48 hours after reperfusion and MBF <50% relative to remote-region blood flow 48 hours after reperfusion ($y=0.8x+0.02$, $r=0.99$).
The extent of microvascular obstruction measured by MRI correlated well with postmortem histopathological examination performed 2 days after infarction and reperfusion.

The magnitude and type of myocardial damage within the infarcted region have been recognized as important determinants of prognosis after acute myocardial infarction. Prolonged coronary occlusion of large epicardial branches led to profound ischemia at the infarct core, resulting in simultaneous necrosis of myocytes and endothelial cells, which would otherwise perish later. This process leads to microvascular obstruction in the infarct core, previously described as the no-reflow or low-reflow region in basic studies and more recently documented in humans by contrast-enhanced MRI and ultrasound.

Previous studies have also documented progression of vascular damage up to 3.5 hours after reperfusion. Whereas Kloner et al. found no differences in the size of no-reflow regions after 90-minute coronary occlusions followed by reperfusion periods of 10 seconds versus 20 minutes, Ambrosio et al. studied canine hearts submitted to 90-minute occlusions but reperfusion for periods of 2 minutes versus 3.5 hours. The areas of absent capillary filling were more extensive after 3.5 hours than after 2 minutes after reperfusion and resulted primarily from intracapillary erythrocyte stasis and marked intravascular neutrophil accumulation.

Recently, contrast-enhanced MRI and echocardiography have provided the opportunity to study the development of microvascular obstruction serially, over many hours or days after coronary occlusion and reflow.

We had previously correlated the presence of MRI hypoenhanced regions at 48 hours after reperfusion with regions of microvascular obstruction documented by radioactive microspheres and thioflavin S staining. Others have demonstrated the presence of microvascular damage by contrast echocardiography and related it to poor functional recovery immediately after infarction. Most recently, we have documented in humans the association between presence of microvascular obstruction by MRI and worse chronic postinfarction long-term prognosis. In that study, we proposed a potential link between the development of profound microvascular damage early after coronary occlusion/reperfusion and eventual ventricular remodeling 6 months to 1 year after myocardial infarction. These architectural changes in ventricular size and function could underlie the worse postinfarction prognosis documented in patients with microvascular obstruction. The present experimental study documents a progressive increase in the extent of no-reflow regions beyond a few hours after reperfusion relative to infarct size. This finding suggests that such a process is dynamic and could theoretically be modulated by intervention early after infarction.

Methodological Considerations

All MRI measurements of infarct size and extent of microvascular obstruction were performed in relation to the MRI-determined total LV mass, whereas histopathological measurements were calculated in relation to postmortem LV mass. Therefore, no quantitative comparisons were performed that used cross-registration of MR images with histopathological data. However, we did cross-register MRI against histopathological data for specific qualitative comparisons of spatial localization of infarcted and no-reflow regions (Figures 3 and 8).

In our study, thioflavin S was used to assess myocardial blood flow as in previous studies. Nonetheless, microvascular occlusion was assessed not only by the thioflavin S but also, and most importantly, by MBF measurements at rest.

**Figure 5.** Time course of microvascular obstruction by contrast-enhanced MRI. Same short-axis image of LV from 1 experiment at 2 hours (A), 6 hours (B), and 48 hours (C) after LAD reflow. These images were acquired during first 3 minutes after Gd-DTPA injection. One can observe an increase in extent of hypoenhanced region (dark region between arrows) over time and accompanying LV remodeling during first 48 hours after infarction/reperfusion.

**Figure 6.** Pooled data from all experiments showing time course of microvascular obstruction measured by MRI (solid columns) and by MBF (open columns). Data are mean ± SEM; P values by repeated-measures ANOVA.
and during dobutamine challenge. We used the thioflavin method for 2 specific reasons: first, to guide MBF determinations by facilitating the identification of boundaries between infarcted no-reflow and infarcted regions, and second, to provide a visual distribution of the extent of microvascular obstruction in the correlation with MRI hypoenhanced regions (Figure 3).

The assessment of microvascular obstruction by MRI is well validated by previous studies. Our results confirm that regions of hypoenhancement by MRI that persist through the first 2 to 3 minutes after the contrast bolus injection reflect microvascular obstruction by both MBF measurements and postmortem staining criteria. In addition, biopsies obtained from MRI hypoenhanced regions in a previous study revealed microvessel obstruction caused by red blood cells and necrotic debris, similar to earlier work characterizing the basic pathophysiology of no-reflow regions after infarction/reperfusion. The ability to relate the extent of microvascular obstruction to infarct size represents an advantage of contrast-enhanced MRI over echocardiography in the study of microvascular integrity in patients with acute myocardial infarction. Thus, the MRI method appears particularly suited for serial clinical studies, given its noninvasive characteristics.

Conversely, the limitations of infarct size measurements as the total hyperenhanced myocardial mass after contrast concentrations reach equilibrium (late hyperenhancement) deserve discussion. Kim et al reported a good correlation between MRI and postmortem TTC infarct size in the isolated rabbit heart model. Similarly, Judd et al reported a good correlation between the total mass of hyperenhanced myocardium and infarct size 48 hours after infarction/reperfusion with methods similar to the ones used in the present study. In the latter study, MRI overestimated infarct size by 12% on average. This is in agreement with results from the present study, which yielded an overestimation of 9%. Findings from other previously reported investigative work have also documented a good correlation between MRI hyperenhancement and infarct size.

The reasons for infarct size overestimation by MRI are not entirely understood. Potential mechanisms include the possibility that myocardial edema without cell necrosis could produce late tissue hyperenhancement through an increase in the volume of distribution for the contrast agent. Theoretically, both intracellular water increase due to cell swelling and interstitial water increase due to plasma infiltration after hyperemia could in part explain our results of progressive

Figure 8. A, Postmortem LV cross section stained by TTC 48 hours after reperfusion. Pale region that failed to stain by TTC (TTC-negative region) represents nonviable infarcted myocardium (between arrows). B, Corresponding MRI image acquired 10 minutes after Gd-DTPA injection showing hyperenhanced region (between arrows), which closely matches TTC-negative region.
increase in total hyperenhanced myocardial mass over time. However, the possibility that reversible myocyte membrane injury would allow intracellular penetration of molecules of Gd-DTPA is still not proved. Although extracellular myocardial edema could have accounted for the MRI infarct size overestimation observed in our experiments (9%), it is an unlikely explanation for our findings of infarct size augmentation, given the substantial increase in total hyperenhancement myocardial mass documented (36.5%) in our study.

Microvascular Flow Reserve

In our study, catecholamine stimulation by dobutamine provides insight into the functional status of the injured microvasculature 48 hours after infarction/reperfusion. MBF measurements during dobutamine infusion showed flow augmentation in all regions except the no-reflow region (thioflavin-negative region). In remote and risk regions (TTC-positive regions underperfused by MBF during coronary occlusion), such flow augmentation was most likely caused by a combination of increased cardiac output and recruitment of microvascular flow reserve induced by increased metabolic demand. The trend toward a lesser dobutamine-induced flow increase in risk compared with remote regions could have been secondary to microvascular “stunning” previously documented as a reduction in microvascular flow reserve (endothelium-dependent vasodilatation) in response to reversible ischemia.25,26

Similarly, in infarcted regions outside the region of microvascular obstruction (TTC-negative but thioflavin-positive regions), dobutamine-induced MBF increase could theoretically reflect partial preservation of microvascular flow reserve, passive blood-flow increase due to increased cardiac output through a nonobstructed microvasculature, or both. However, given previous work documenting a loss of microvascular flow reserve within infarcted regions outside the no-reflow zone,7,10 the second mechanism probably underlies our findings of MBF augmentation in those regions. Conversely, in thioflavin-negative regions, the absence of dobutamine-induced flow increase 48 hours after reperfusion reflects both microvascular obstruction and loss of recruitable microvascular flow reserve.

Potential Mechanisms of Progressive Myocardial and Microvascular Damage After Infarction/Reperfusion

The mechanisms of progressive microvascular obstruction beyond coronary occlusion and reflow are not completely understood. Our results indicate that either cellular death at the microvessel level is already established during the ischemic insult, unfolding after arterial reflow and reperfusion, or some injurious mechanism(s) continues or begins to operate during the reperfusion process.

The hypothesis that the final extent of microvascular obstruction is determined during the occlusion period has been proposed and is supported by several previous observations.2,28 By contrast, the hypothesis that the additional microvascular and myocyte damage that occurs after the onset of reperfusion is caused by mechanisms activated after coronary artery reflow has also been proposed and is supported by a number of other previous studies.16,29 Potential mechanisms include direct endothelial damage through oxygen radical formation7,30, altered vascular reactivity25,27, progressive intracapillary erythrocyte and/or granulocyte accumulation, causing mechanical microvessel obstruction7,30,31, and progressive myocyte swelling, which may lead to continued microvascular compression.28,30,32,33 Although our results do not provide specific support for either of the 2 alternative hypotheses mentioned above, they do demonstrate progressive microvascular impairment and myocyte damage well beyond coronary artery recanalization. Such knowledge is important to our understanding of the fundamental processes that underlie acute myocardial infarction, particularly given the link between microvascular obstruction and ventricular remodeling both experimentally34 and clinically.6,22

Conclusions

Our study documents progressive microvascular obstruction within infarcted territory beyond coronary reflow up to 48
hours after myocardial infarction. This increase in the no-reflow region relative to infarct size was documented by both MRI and MBF methods. In addition, we also report progressive augmentation in the total myocardial mass injured by ischemia/reperfusion up to 48 hours after myocardial infarction. These results support the concept that myocardial injury continues beyond reperfusion, in terms of both additional microvascular damage and total infarcted myocardial mass.

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