Characterization of Spatial Patterns of Flow Within the Reperfused Myocardium by Myocardial Contrast Echocardiography

Implications in Determining Extent of Myocardial Salvage

Flordeliza S. Villanueva, MD; William P. Glasheen, PhD; Jiri Sklenar, PhD; Sanjiv Kaul, MD

Background. Since myocardial blood flow changes dynamically after reperfusion and since both hyperemia and impairment in microvascular function exist within the acutely reperfused bed, we sought to investigate the role of myocardial contrast echocardiography (MCE) in (1) defining the temporal variability in perfusion patterns after reflow and relating these to microsphere-derived blood flow; (2) differentiating viable from infarcted tissue during different periods of reflow; and (3) defining spatial perfusion patterns within the infarct bed in response to exogenously induced maximal vasodilation and relating these to infarct size and extent of myocardial salvage.

Methods and Results. Twenty-one dogs with 3 hours of left anterior descending coronary artery occlusion and 2 to 3 hours of reflow were studied. MCE was performed at 15 and 45 minutes and 2 and 3 hours after reflow. It was also performed at either 2 or 3 hours after reflow in the presence of 0.56 mg/kg of dipyridamole. Radiolabeled microsphere-derived blood flow was measured at 15 minutes and 2 and 3 hours after reflow and during dipyridamole effect. Infarct size was measured at the end of the experiment by use of triphenyl tetrazolium chloride. MCE data were processed with color-coding schemes that highlighted differences in myocardial videointensities in proportion to the concentration of microbubbles within the microvasculature. There was significant variability in MCE-defined perfusion patterns after reflow, with contrast defects noted mainly within the endocardium. There was fair and significant (P<.05) correlation (r = −.73 to r = −.55) between MCE defect size and normalized endocardial blood flow. Except at 15 minutes after reflow, there was poor correlation (r = .31 to r = .51) between MCE defect and infarct sizes. Even at 15 minutes after reflow, MCE defect size underestimated infarct size by 50%. In comparison, in the presence of dipyridamole, MCE defect size correlated strongly (r = .87, P <.001) with infarct size and reasonably well with normalized transmural blood flow (r = −.62, P = .04). Moreover, the topography of the MCE perfusion defect reflected the topography of the infarct.

Conclusions. MCE revealed striking temporal heterogeneity in the spatial distribution of myocardial perfusion during postsischemia reflow and either significantly underestimated or did not correlate with infarct size during reperfusion. Because of abnormalities in coronary vascular reserve specific to infarcted tissue, MCE in conjunction with intravenous dipyridamole depicted, in vivo, the actual topography of the infarct with remarkable accuracy. (Circulation. 1993;88:2596-2606.)

Key Words • myocardium • blood flow • reperfusion • echocardiography

When anterograde blood flow is restored to an occluded coronary artery in patients with acute myocardial infarction, it is generally presumed that flow is also restored to the myocardium. This belief has led to attempts at correlating short- and long-term clinical outcome with vessel patency at different intervals after attempted reflow. By and large, clinical outcome has not been correlated with the extent of myocardium that has been reperfused despite ample experimental evidence that flow is not always restored to all myocardial regions even though reflow to the infarct-related artery has been achieved.1-6

Using myocardial contrast echocardiography (MCE), Ito and colleagues7 recently demonstrated that myocardial perfusion was markedly reduced in approximately one fourth of their patients with acute myocardial infarction despite angiographic documentation of reflow in the infarct-related artery within 6 hours of onset of symptoms. These authors observed that patients showing lack of myocardial perfusion despite having a patent artery had worse regional and global left ventricular function 1 month later than those who showed myocardial perfusion within the infarct bed.

MCE uses intracoronary injection of microbubbles of air that delineate the spatial distribution of blood flow within the myocardium.8,9 Since there is sustained hyperemia within the infarct bed for several hours after
reperfusion, it would be expected that using markers of flow such as microbubbles of air would result in underestimation of the degree of myocardial necrosis and hence, overestimation of the degree of myocardial salvage. Why then did Ito and colleagues observe a relation between perfusion patterns at the time of reflow and regional and global left ventricular function 1 month later? Similarly, perfusion patterns have been demonstrated to change dynamically within the first few hours to days after reflow. Consequently, at what interval after reflow would the extent of myocardial salvage optimally be determined? Finally, despite hyperemia within the infarct bed, microvessel function is dramatically altered, with necrotic regions showing the most prominent changes, including the most compromised flow reserve. Since MCE can assess microvascular patency and reserve could it be used after reflow to define myocardial regions with necrosis that either do not have patent microvessels or, if the microvessels are patent, their reserve is significantly altered? Similarly, could it be used to define salvaged myocardium that has patent microvasculature with minimal alterations in the microvascular reserve?

The present study was undertaken to address these issues using a canine model of sustained coronary occlusion followed by reflow. We sought to determine whether MCE could be used to (1) reflect dynamic changes in regional myocardial perfusion during postischemia reflow; (2) differentiate viable from infarcted tissue during reflow; and (3) relate spatial perfusion patterns within the infarct bed in response to exogenously induced maximal vasodilation to infarct size and the extent of myocardial salvage.

Methods

Animal Preparation

Twenty-one dogs were used in a model of 3 hours of coronary occlusion followed by 2 to 3 hours of reperfusion. The protocol conformed to guidelines for animal research use at the University of Virginia. The dogs were anesthetized with 30 mg/kg sodium pentobarbital (Abbott Laboratories, North Chicago, Ill), intubated, and ventilated with a respirator pump (model 607, Harvard Apparatus, Natick, Mass). Additional anesthesia was administered during the experiment as needed. A 7F catheter was placed in the right femoral artery for recording of arterial pressure and withdrawal of reference samples for radiolabeled microsphere analysis. This catheter was connected to a multichannel recorder (model 4568C, Hewlett Packard, Everett, Mass) via a fluid-filled transducer (model 1280C, Hewlett Packard). Another 7F catheter was placed in the left femoral vein for intravenous administration of drugs and fluids as needed.

A left lateral thoracotomy was performed, and the heart was suspended in a pericardial cradle. A 7F catheter was placed in the left atrium for injection of radiolabeled microspheres (Fig 1). The proximal to mid portion of the left anterior descending coronary artery was isolated, and a tie with a snare was loosely placed around it. A 3.5-cm-long 21-gauge polyethylene catheter was inserted retrogradely through the anterior wall of the left anterior descending coronary artery, and its tip was positioned in the left main artery (Fig 1). The left main placement was confirmed by injecting contrast into the catheter and obtaining homogeneous opacification of the entire left ventricular myocardium. The proximal end of the catheter was connected to a power injector (model 3000, Liebel-Flarsheim Co, Cincinnati, Ohio) for administration of microbubbles.

Myocardial Contrast Echocardiography

MCE was performed with a phased array system (RTS5000, General Electric Medical Systems, Milwau-

kee, Wis) with a 5-MHz transducer. Gain settings were optimized initially and held constant throughout the experiment, and a maximal dynamic range of 72 dB was used. A saline bath served as an acoustic interface between the heart and the transducer. Imaging was performed at the mid papillary muscle short-axis level, and care was taken to record the same imaging plane at each stage of the experiment to allow serial comparisons within each dog. Data were recorded on 1.25-cm VHS videotape with a high-fidelity video recorder (Panasonic model AG6200, Matsushita Electrical Co, Japan).

Sonicated albumin microbubbles (AlbuneX, Molecular Biosystems Inc, San Diego, Calif) with a mean size of 4.3 μm and a concentration of 0.5 billion bubbles per milliliter were used as the contrast agent. This agent has been demonstrated to cause no changes in systemic and coronary hemodynamics when injected directly into the coronary artery in doses required to achieve myocardial opacification. At each stage of the experiment, MCE was performed by power injection of 0.5 mL of this agent into the left anterior descending coronary artery during simultaneously performed echocardiographic imaging. Pilot studies demonstrated that this dose produced optimal myocardial opacification without attenuation. In this study, we opted to inject contrast directly into the coronary circulation rather than into the left or right atrium to maximize the signal-to-noise ratio and avoid attenuation of the posterior wall, which occurs consequent to the presence of microbubbles in the left ventricular cavity in the latter situations.

MCE images were analyzed with an off-line computer (Mipron, Kontron Electronics, Germany) as previously described. The images were transferred from videotape to image memory of the computer in a 244×244×8-bit format. Consecutive end-diastolic frames, encompass-
ing the period from just before contrast injection until 10 seconds thereafter, were selected and aligned by computer cross-correlation techniques.\textsuperscript{22} The aligned images were processed by a method designed to optimally define perfusion defect borders.\textsuperscript{23,24} Three precontrast end-diastolic frames were averaged to improve the signal-to-noise ratio, and three contrast-enhanced end-diastolic frames were similarly averaged. The averaged precontrast frame was digitally subtracted from the averaged postcontrast frame. Videointensities in the subtracted image were expanded to a dynamic range of 128 levels; i.e., the brightest pixel was assigned a value of 128 and all other pixels were assigned lower values on a linear scale. Reassigned values of $<10$ were considered to represent noise. Each pixel with a gray scale assignment of $>10$ was relegated a color, whereby gradations of red to orange to yellow to white represented increasing degrees of contrast enhancement. Pixels showing a change of $<10$ in the assigned gray scale of 128 levels were not encoded with color. During coronary occlusion, myocardial regions with absent color represented risk area, whereas during reperfusion, areas with relative color deficiency represented regions of reduced flow. These areas were planimetered and expressed as a percentage of the myocardium in the short-axis slice. This approach has previously been shown to accurately delineate functional myocardial risk area during coronary occlusion.\textsuperscript{9,25}

**Determination of Infarct Size**

Postmortem, the heart was sectioned into slices 1 cm thick perpendicular to its long axis. The slice corresponding to the MCE image was immersed in a solution of 1.3% 2,3,5-triphenyl tetrazolium chloride (TTC) and 0.2 mol/L Sorensen’s buffer (KH$_2$PO$_4$ and K$_2$HPO$_4$ in distilled water, pH 7.4) at 37°C for 20 minutes and then fixed in 10% formalin.\textsuperscript{9,26} This histochemical technique stains viable areas brick red, whereas infarcted areas remain unstained.\textsuperscript{26} Images of the basal and apical sides of the TTC-stained slice were captured into the off-line computer by a video camera with a resolution of 600 lines per field (66 series, Dage-MTI Corp, Michigan City, Ind).\textsuperscript{9} Infarct size was determined by planimetering the unstained portions of the basal and apical sides of the specimen, taking their average, and expressing this as a percentage of the short-axis slice or MCE-determined risk area.\textsuperscript{9}

**Myocardial Blood Flow Measurements**

Approximately 2$x10^6$ 11-μm microspheres (Dupont Medical Products, Wilmington, Del), suspended in 4 mL of 0.9% saline solution/0.01% Tween 80, were injected into the left atrium at each stage. Reference samples were withdrawn from the femoral artery over a period of 90 seconds with a constant-rate withdrawal pump (model 944, Harvard Apparatus). The short-axis slice of the left ventricle corresponding to the MCE image was cut into 16 wedge-shaped segments after TTC staining, and each piece was divided into endocardial, midwall, and epicardial portions. Papillary muscles were included in the endocardial portions. The tissue and arterial reference samples were counted in a well counter with a multichannel analyzer (model 5986, AutoGamma Scintillation Counter, Packard Corp, Downer’s Grove, Ill). Corrections were made for activity spilling from one window to the next with a custom-designed computer program.\textsuperscript{8} Flow to each sample was calculated from the equation $Q_m = (C_m - C_i)/C_i$, where $Q_m$ is myocardial flow (in milliliters per minute), $C_m$ is tissue counts, $Q_i$ is rate of arterial sample withdrawal (in milliliters per minute), and $C_i$ is counts in the arterial reference sample.\textsuperscript{27} Transmural blood flow (in milliliters per minute per gram) was derived by dividing the sum of flows to the individual segments by their combined weight. Blood flow was measured in the risk area (excluding the lateral borders with intermediate flow) and normalized to flow in the nonischemic posterior wall.

**Experimental Protocol**

The left anterior descending coronary artery was occluded for 3 hours, toward the end of which MCE was performed to assess risk area. After release of the occlusion, reperfusion was maintained for 2 hours in all 21 dogs and an additional 1 hour in 17 dogs. MCE was performed in all dogs at 15 and 45 minutes and 2 hours after reperfusion, and in 17 dogs, it was also repeated at 3 hours. The last MCE assessment in each dog was followed by another MCE assessment 2 minutes after the intravenous infusion of 0.56 mg/kg of dipyridamole. This maneuver was performed to elicit perfusion patterns after exogenously induced maximal coronary vasodilation. Radiolabeled microsphere blood flow measurements were performed at 15 minutes and 2 and 3 hours in most dogs, and in a smaller subset they were also performed after the intravenous administration of dipyridamole. At the end of the experiment, the dog was killed, and the slice of the heart corresponding to the MCE image was processed to determine infarct size and myocardial blood flow.

**Statistical Methods**

Data were expressed as mean±SD. Correlations between MCE-derived and TTC-derived or radiolabeled microsphere–derived measurements were made by linear regression analysis. Statistical significance was defined as $P<.05$ (two-sided).

**Results**

**MCE Perfusion Patterns**

MCE data suitable for quantitative analysis were obtained at 15 minutes and 2 hours after reflow and after dipyridamole infusion in all 21 dogs and at 45 minutes and 3 hours in 17 dogs. The risk area ranged from 22% to 59% of the left ventricular short-axis slice, and infarct size ranged from 13% to 86% of risk area. MCE images indicated that there was temporal heterogeneity in the spatial distribution of myocardial blood flow during postischemia reperfusion. Figs 2 through 4, taken from dogs with infarctions of varying transmurality, illustrate the evolution of myocardial contrast perfusion patterns during reflow as well as the ability of MCE in the presence of dipyridamole to delineate actual infarct topography.

Fig 2 depicts changes in perfusion patterns after reflow in a dog with a nearly transmural infarction. MCE after 45 minutes of reperfusion demonstrated a small relative color defect localized to the endocardium in the anterior wall as depicted by arrows in panel A.
After 3 hours of continuous reperfusion, this defect was slightly larger, as shown by arrows in panel B. The addition of dipyridamole resulted in a much larger relative contrast defect, depicted by arrows in panel C, which closely delineated the true size and shape of the infarct as defined by TTC staining and identified by arrows in panel D.

Fig 3 exemplifies serial contrast defects in a dog with a moderate-sized infarct localized to the endocardial half of the anterior myocardium. MCE after 15 minutes of reperfusion demonstrated a relative contrast defect involving almost the entire myocardial thickness of the anterior wall, as depicted by arrows in panel A. After 3 hours of reperfusion, almost no defect was seen, as shown in panel B, although the anterior wall had relatively less perfusion than the posterior wall, since it exhibited only hues of red, whereas the posterior half of the myocardium had hues of yellow. With the administration of dipyridamole at 3 hours, the region with no color, indicated by arrows in panel C, closely paralleled the size and distribution of the infarct delineated by TTC staining in panel D.

Fig 4 illustrates sequential MCE images in a dog with minimal infarction located within the endocardial surfaces of the two heads of the anterior papillary muscle. A small patchy defect was seen in the anterior myocardium 15 minutes after release of the occlusion, as shown in panel A. At 3 hours, the defect was even smaller or negligible, as depicted in panel B. The addition of dipyridamole resulted in a relative color defect, indicated by arrows in panel C, which corresponded in location with the papillary muscle infarct seen on TTC staining depicted by arrows in panel D.

Serial MCE data in all 21 dogs are summarized in Fig 5. Within each dog, there was marked temporal variability in the size of the perfusion defect during reflow, with severalfold decreases or increases in defect size during the reperfusion period. Interestingly, as illustrated in Figs 3 and 4, defects early after reflow generally tended to be larger than subsequent defects.

Relation Between MCE Defects and Microsphere-Derived Blood Flow

MCE defects, which were predominantly endocardial, reflected the status of regional myocardial blood flow during reperfusion. Fig 6 shows the relation between defect size and normalized endocardial blood flow in the dogs with simultaneous microsphere and MCE data at each stage. At 15 minutes (panel A), 2 hours (panel B), and 3 hours (panel C) after reperfusion, MCE defect size was generally related to endocardial blood flow, with relatively low flows resulting in larger MCE defects and high flows resulting in smaller MCE defects. No defects were usually seen when endocardial flows were high (>70% of flow in the normal bed). As would be expected, transmural flows after
reperfusion did not correlate well with defect size, since these were predominantly endocardial in most dogs and the epicardial regions exhibited relative hyperemia.

The variability in MCE defect size in individual dogs shown in Fig 5 paralleled dynamic changes in blood flow to the risk area during reperfusion, as illustrated in Fig 7. At 15 minutes after reperfusion, transmural flow to the postischemic bed was attenuated in half the dogs and hyperemic in the other half, with the result that mean flow was similar to that in the normal bed. The dogs showing reduced transmural flows had predominantly low endocardial flows. The fluctuations in flows subsided over time, but despite infarction, the mean transmural flow in all dogs remained high within the postischemic bed. Even within the endocardium, in which most infarcts were located, absolute flows remained relatively high at 3 hours after reflow, ranging from 0.4 to 2.5 mL·g⁻¹·min⁻¹.

As with serial MCE (Fig 5), each dog demonstrated temporal variability in flow to the risk zone during reperfusion, with as much as a threefold increase or twofold decrease in transmural flow between consecutive stages (Fig 7). Furthermore, in dogs with low initial endocardial perfusion (normalized flow of <1 at 15 minutes of reperfusion), flow increased significantly by 2 hours of reperfusion (0.44±0.39 to 0.85±0.53, n=11, P=.004) and remained similar at 3 hours. Interestingly, this temporal trend of increasing endocardial flow tracked the MCE data, in which MCE defects were largest at 15 minutes and smaller thereafter (Fig 5).

Relation Between MCE Defects and Infarct Size

MCE defect size and the extent of infarction delineated by TTC staining were sporadically related during the reperfusion period, as shown in Fig 8. For instance, at 15 minutes of reperfusion, infarct and MCE defect size were linearly related, but the MCE defect consistently underestimated infarct size by approximately 50% (panel A). At 45 minutes (panel B) and 2 hours (panel C) of reperfusion, MCE did not predict infarct size, whereas at 3 hours of reperfusion (panel D), the MCE defect and infarct size were again statistically related, but the correlation coefficient was poor, and the MCE measurements markedly underestimated infarct size. Notably, at all time points during reperfusion, there were frequently minimal or no contrast perfusion defects over a wide range of infarct sizes.

The microsphere data were consistent with these discrepancies between MCE defect size and extent of infarction. Regional myocardial blood flow to the risk area during reperfusion was related to the extent of myocardial necrosis, as with MCE, only at 15 minutes of reflow. There was an inverse linear relation between normalized endocardial blood flow at 15 minutes of reperfusion and TTC-defined infarct size expressed as a percentage of risk area (y=0.26x+0.70, r=.72, SEE=0.16, P<.002). There was no such relation, however, at any of the other time points assessed during reperfusion. Thus, the extent of microsphere-derived "low reflow," as with the MCE images, predicted the extent of infarction only at 15 minutes after release of the coronary occlusion.

In comparison, in the presence of dipyridamole, MCE defects more accurately approximated the infarct size (Figs 2 through 4, panel C). Unlike the situations in Fig 8, perfusion defect size was closely related to infarct size and only marginally underestimated it (Fig 9). Because of the imaging algorithm used, in which pixels were assigned gray scale and color values only in reference to the brightest pixels, dipyridamole was able to effect a marked relative reduction in transmural perfusion within the risk area compared with the posterior bed (Fig 7).

After dipyridamole infusion, because of reduced microvascular reserve, flow did not increase in the infarct bed as much as in the normal bed, being only 33±20% of that in the normal bed. This pattern was confirmed in the 11 dogs with both MCE and microsphere data during dipyridamole infusion (Fig 10), in which larger MCE defects corresponded to the lowest transmural flows and smaller defects to relatively higher flows in the postischemic bed. Unlike the 15- and 45-minute and 2- and 3-hour images, in which perfusion defect size correlated only with endocardial flow (since in these situations they were located mainly within the endocardium) and not with transmural flow, after dipyridamole infusion, perfusion defect size correlated more closely with transmural blood flow, and the defects themselves exhibited a greater degree of transmularity.

Discussion

This study used the relative presence or absence of myocardial contrast enhancement during reflow to seri-
ally delineate the spatial distribution of myocardial perfusion in mixed infarcted and viable postischemic tissue. With this approach, MCE revealed striking temporal heterogeneity in the spatial distribution of myocardial perfusion patterns during postinfarct reflow, which corresponded well with actual blood flow measurements to the infarcted endocardium. MCE either significantly underestimated or did not correlate with infarct size during reperfusion. Furthermore, because of abnormalities in microvascular reserve specific to infarcted tissue, MCE in conjunction with intravenous dipyridamole depicted, in vivo, the actual topography of the infarct with remarkable resolution and accuracy.

**Detection of Myocardial Infarction During Postischemia Reperfusion**

In the absence of pharmacological vasodilatation, MCE during reperfusion could not accurately quantify myocardial infarction. Microsphere data indicate that these findings were a result of significant levels of flow to the infarct during the first few hours of reperfusion, a phenomenon that has also been shown by others who used radiolabeled microspheres.\(^5,10,11\) In myocardial segments that were >90% necrotic on microscopic analysis, for example, Cobb et al\(^1\) found a onefold to twofold increase above control in radiolabeled microsphere-derived blood flow shortly after reperfusion. Although this degree of reactive hyperemia is less than would be expected for noninfarcted tissue experiencing similar levels of ischemia,\(^11,28,29\) and although patches of necrotic tissue do manifest true “no-reflow” conditions,\(^1,10\) even such flow may be quantitatively sufficient to be seen on MCE, which can detect contrast enhancement from intracoronary injections at flows as low as 0.15 mL·g\(^{-1}\)·min\(^{-1}\).\(^8\)

It is for this reason that we used measures of relative degrees of contrast enhancement (encoded in color) to better discern regions with relatively low flow. Nonetheless, MCE performed up to 3 hours after reperfusion did not predict actual infarct size. It was only at 15 minutes of reflow that MCE defect size or microsphere-derived blood flow bore any systematic relation to infarct size. Even then, MCE underestimated the actual extent of infarction. Kemper and colleagues\(^30\) have also reported the underestimation of infarct size using MCE during reflow.

**MCE and the ‘No-Reflow’ Phenomenon**

Although MCE could not precisely delineate infarction in the absence of dipyridamole, areas with the least flow during reperfusion were located predominantly in the endocardium, in which the extent of necrosis is most
Temporal Variability in Postischemic Perfusion

Because MCE can be performed repeatedly, this study graphically depicted the dynamic nature of "low reflow," which has heretofore been difficult to characterize in vivo. Our data indicate that there is temporal variability in the spatial distribution of regional myocardial perfusion within the first few hours after reflow. Unlike other studies, our study did not find a progression in the spatial extent of the "no-reflow" phenomenon during reperfusion, which may be partly because of differences in experimental design, the duration of coronary occlusion, and methods used to assess "no reflow." Ambrosio et al. for instance, used dogs undergoing only 90 minutes of occlusion and either 2 minutes or 3.5 hours of reperfusion and found a three-fold increase in the "no-reflow" phenomenon in the group of dogs subjected to the longer period of reperfusion. The dye used by Ambrosio et al to detect "no reflow" failed to stain at flows <0.4 mL·g⁻¹·min⁻¹, which is a higher threshold for detecting "no reflow" than for MCE.

Microvascular Reserve in Postischemic Myocardium

MCE at 2 or 3 hours of reperfusion, in the presence of pharmacological vasodilation, was able to delineate with excellent resolution the actual topography of the myocardial infarct. The microsphere data confirm that during dipyridamole administration, myocardial blood flow is redistributed to tissue with intact coronary reserve—the viable epicardium and posterior bed—resulting in relative hypoperfusion and a relative paucity of bubbles entering the infarcted myocardium. The latter results in an area of relative contrast deficiency.
resembling the infarction. These results are consistent with reports of others.\textsuperscript{2,3} In homogeneously infarcted endocardium, for instance, Vanhaecke et al\textsuperscript{3} have demonstrated an absence of vasodilator response to adenosine infusion.

Our data also support the observations of Johnson et al,\textsuperscript{2} who found that myocardial damage was necessary to detect impairments in vascular conductance in a post-ischemic bed and that there was a relation between infarct size and transmural blood flow only in the presence of exogenously induced vasodilation. These previous studies, however, could not ascertain whether the decrease in flow during pharmacological vasodilation was caused by microvascular impairments in the infarcted tissue or in the entire risk area. The present study indicates that the decrease in conductance or flow in response to exogenously administered vasodilators is caused by a marked decrease in flow exclusively within the infarct borders.

The mechanisms underlying these vascular impairments during reperfusion remain to be elucidated. Data suggest that a combination of structural, mechanical, and/or functional abnormalities at the microcirculatory level (or even the epicardial coronary artery level) probably result not only from reperfusion but also from ischemia itself.\textsuperscript{2,4,6,14-16,32-34} These derangements may ultimately manifest as dynamic shifts in patterns of "low reflow" with time, abnormal augmentation of vascular tone, or inappropriate responses to pharmacological vasodilation. Any of these processes or a combination would affect the microcirculatory transit of a flow tracer, such as microbubbles, and could, therefore, be detected by MCE.

Critique of Our Methods

The delineation of defects was highly dependent on the color-coding algorithm used, whereby all pixels in the myocardium are scaled in relation to the brightest pixel. This approach was used to improve the detection of infarcted areas with persistent but relatively low flow, particularly during the dipyridamole effect, but relied on operator judgment to determine the borders of the perfusion defect amid a continuum of color changes. Nevertheless, the borders of relatively confluent infarcts were identified with a high degree of accuracy compared with TTC staining. Whether MCE can spatially resolve small patchy infarcts conglobed with viable tissue, or areas with localized inhomogeneity in microvascular reserve,\textsuperscript{38} remains to be demonstrated.

Although slight movement of the MCE imaging plane between stages could have accounted for some of the temporal variability in the data, we do not believe that this was the case. Caution was taken to obtain the same imaging plane for each experimental stage by reviewing data from the preceding stage, and as Figs 2 through 4 demonstrate, the serial images were essentially identical. Also, the comparable temporal variability in the microsphere data and their significant relation between MCE defects at each time point confirm that the variability in the MCE defects was a result of true changes in flow.

We used peak videointensity to gauge relative myocardial blood flow during MCE, which at any given time relates to the concentration of microbubbles within tissue. When myocardial blood flow is increased by endogenous (postischemia) or exogenous (dipyridamole or adenosine) vasodilation, in the absence of changes in systemic hemodynamics this increase is related to increase in myocardial blood volume.\textsuperscript{20} When contrast is injected in this situation, more bubbles are present within each unit of tissue, resulting in an increase in peak videointensity. Since we measured only relative videointensity, it was not necessary to have a strictly linear response between microbubble concentration and videointensity, as long as more bubbles produced higher peak intensities.

Our results could be specific to the model used. For instance, we used a protocol in which the occlusion was completely reversed. Whether similar results can be obtained with a persistent coronary stenosis during reflow remains to be documented. It is possible that the hyperemia noted in the epicardium that is necessary to discriminate between infarcted and noninfarcted tissue during exogenous coronary vasodilation may be variably
attenuated in the presence of severe stenosis. Similarly, whether identical results can be obtained in the setting of multivessel disease and what role the presence or absence of collateral blood flow could have on our findings remain to be investigated.

**Clinical Implications**

After attempted reperfusion, it would be useful to know how much of the myocardium has been salvaged. Although extrapolation of experimental observations into the clinical setting should be made with caution, the results of the present study support our uncontrolled observations using MCE in patients with acute myocardial infarction. These results also explain why Ito and colleagues'7 were able to correlate perfusion patterns 15 minutes after reflow with regional function 1 month later. As stated earlier, it was only at 15 minutes after reflow that we found a reasonable correlation between perfusion defects on MCE and infarct size. At 45 minutes and 2 and 3 hours after reperfusion, the

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**Fig 8.** Scatterplots showing relation between perfusion defect size defined on myocardial contrast echocardiography (y axis) and triphenyl tetrazolium–defined infarct size (x axis) at 15 minutes (A), 45 minutes (B), 2 hours (C), and 3 hours (D) after reflow. The dotted line in each panel depicts the line of identity. See text for details.

**Fig 9.** Scatterplot showing relation between perfusion defect size defined on myocardial contrast echocardiography (y axis) and triphenyl tetrazolium–defined infarct size (x axis) after intravenous infusion of 0.56 mg/kg dipyridamole. Unlike the situations in Fig 7, perfusion defect size is closely related to infarct size and only marginally underestimates it. See text for details.
correlation was much poorer. Because of hyperemia, even at 15 minutes after reperfusion, defect size on MCE significantly underestimated infarct size and thus overestimated the extent of myocardial salvage. It is conceivable that had Ito and colleagues performed MCE later after reflow, their results would not have been as striking.

Our results also indicate that during reperfusion, microvascular reserve is reduced within the infarcted tissue despite relative hyperemia. Performing MCE after the intravenous infusion of dipyridamole can demarcate these regions of reduced microvascular reserve and, in doing so, define the respective topographies of necrotic and viable tissue within the infarct bed with remarkable resolution. Obviously, our results pertain to open-chest preparations, and it may not be possible to separate necrotic from viable tissue with the same degree of accuracy using transthoracic echocardiography.

Although in this study we used dipyridamole at 2 to 3 hours after reflow, there is no reason to believe that its use 15 minutes after reflow would not yield similar results. Using a preparation of 3 to 6 hours of coronary occlusion followed by 15 minutes of reflow, we were able to demonstrate an excellent relation between MCE contrast defect size and infarct size during dipyridamole infusion after left and right atrial injections of contrast. Dipyridamole has been used safely in patients with chronic coronary artery disease. Preliminary results also attest to the safety of this agent in patients within 1 to 3 days after acute myocardial infarction. Further studies are needed to determine the safety of this agent or other coronary vasodilators in the first few hours after an acute myocardial infarction.

A coronary vasodilator that can be used safely in the early reperfusion period coupled with technical advances in microbubble engineering and ultrasound devices may make it possible to depict the extent of myocardial salvage after attempted reperfusion using MCE. Knowledge of the size and location of risk area during coronary occlusion and the extent of myocardial salvage immediately after attempted reperfusion could have major value in managing patients with acute myocardial infarction. Furthermore, a method that can assess myocardial microvascular patency and reserve could also aid in determining pharmacological and other strategies for attenuating microvascular injury during acute myocardial infarction.

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**Fig 10.** Scatterplot showing relation between perfusion defect size on myocardial contrast echocardiography (depicted on the y axis) and transmural blood flow within the risk area normalized to that in the normal posterior wall after dipyridamole infusion (x axis). Unlike the 15- and 45-minute images and 2- and 3-hour images in which perfusion defect size correlated only with endocardial flow (since in these situations they were located within the endocardium) and not with transmural flow, after dipyridamole infusion, perfusion defect size correlated more closely with transmural blood flow and exhibited a greater degree of transmurality. See text for details.


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