Assessment of Myocardial Postreperfusion Viability by Intravenous Myocardial Contrast Echocardiography  
Analysis of the Intensity and Texture of Opacification

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Background—Although defects on intracoronary myocardial contrast echocardiography (MCE) indicate loss of viability after reperfusion, opacified segments may also exhibit persistent dyssynergy. Therefore, we related the intensity and texture of opacification produced by an intravenous contrast agent to histological findings to determine the characteristics of necrotic tissue by postreperfusion MCE.

Methods and Results—MCE was performed by intravenous injection of 0.15 mL/kg QW7437 in 14 dogs who underwent 3-hour coronary occlusion followed by 3-hour reperfusion. At baseline and 3 hours after reperfusion, midventricular short-axis images were digitized and segmented. Infarction fraction (IF) for each segment was determined by triphenyltetrazolium chloride stain. Of 224 segments, 140 showed no or small infarction and served as a control group. Of 84 segments with significant infarction (IF > 30%), 52 exhibited a defect on MCE, and 32 exhibited no defect. Echo texture was quantified by computing entropy based on the co-occurrence matrix analysis of gray-level pairs within each segment. Three hours after reperfusion, average and maximal entropies in the infarct segments without opacification defects were significantly higher than control levels. Histologically, the degree of intracapillary erythrocyte stasis was less in this group than in the infarcted segments with MCE defects with similar magnitude of tissue injuries.

Conclusions—Opacification defects by MCE may be present or absent in myocardium with histologically confirmed infarction. The texture of MCE from opacified but infarcted myocardium differed significantly from control segments and may assist in determination of segmental viability after reperfusion. (Circulation. 2001;103:2021-2027.)

Key Words echocardiography ■ reperfusion ■ coronary disease ■ myocardial infarction

It has been proposed that myocardial contrast echocardiography (MCE), by virtue of its ability to assess microvascular integrity, may serve as a marker of successful reperfusion of acute myocardial infarction.1-7 From MCE produced by direct coronary injection, patterns of opacification consisting of absent, partial/patchy,3,6 and normal uptake1-7 have been observed in the reperfused zone shortly after recanalization. The absence of myocardial opacification after reperfusion has been associated with necrosis, as evidenced by a failure to recover function at follow-up.1 Patchy opacification has been accompanied by partial recovery of contractile function and has been considered to represent a mixture of infarcted and viable myocardium.3 Dense contrast enhancement in the risk area has generally indicated viable myocardium.1

Although the relationship of the foregoing patterns of contrast enhancement to the status of myocardial viability or necrosis have generally held true, several important issues remain unanswered. Few data exist regarding the histological findings manifested by myocardial segments with various degrees of opacification by MCE after reperfusion. Although previous studies have applied intravenous contrast to study infarction,4 the distribution of new contrast agents capable of myocardial enhancement by intravenous injection has not been fully defined after restoration of flow in an occluded coronary artery. Of greatest importance, it has been found that myocardial dysfunction may occur even in the presence of contrast enhancement4-6 and that an additional assessment of the response to dobutamine stress may be necessary to establish viability.6,7 Thus, alternative methods by which to identify infarcted but opacified myocardium after reperfusion are needed.

With the foregoing issues in mind, we examined the relationship between the opacification pattern produced by a new intravenous dodecafluoropentane contrast agent and histological evidence of necrosis or viability after reperfusion.
of coronary occlusion. Myocardial echo texture analysis was applied to this problem because this technique has been shown to be capable of identifying various myocardial disorders.9–11 We reasoned that the patchy appearance frequently seen in opacified but infarcted regions might be identified by texture analysis, which quantifies spatial distribution pattern of gray levels (GLs) in echocardiograms.

Methods

Experimental Preparation
The present study was approved by the Animal Research Committee at the University of California at San Diego and conformed to the “Position of the American Heart Association on Research Animal Use” (Circulation. April 1985). Fourteen mongrel dogs (22.3 ± 2.0 kg) were anesthetized with 30 mg/kg sodium pentobarbital and ventilated to keep arterial blood gases and pH within normal limits.

The right femoral artery and vein were cannulated for arterial pressure monitoring and contrast agent injection, respectively. The heart was exposed through a left lateral thoracotomy and suspended in a pericardial cradle. The proximal portion of the left anterior descending coronary artery was dissected free.

Myocardial Contrast Echocardiography
MCE was performed by an intravenous injection of QW7437 (SONUS Pharmaceuticals). This agent is a dodecafluoropentane emulsion that produces microbubbles with negative surface charge12 after hypobaric activation. We drew 0.15 mL/kg of QW7437 into a 30-mL syringe and applied hypobaric and shock-wave activation by rapid negative suction12 immediately before bolus injection, which was followed by a 5-mL saline flush. QW7437 has been observed to exhibit prolonged myocardial opacification after intravenous bolus injection.12

Echocardiography was performed with a broadband frequency transducer (4 to 7 MHz) (HDI-3000, ATL). A latex bag filled with degassed water was placed on the anterior wall of the left ventricle to provide an acoustic standoff between the transducer and the heart. The transducer was positioned to yield optimal images and was held constant by a mechanical holder.

Short-axis images of the left ventricle at the midpapillary muscle level were obtained with the fundamental mode and triggering on the R wave of the ECG and were recorded on S-VHS videotape. We used a mechanical index of 0.6, linear compression for postprocessing, a dynamic range of 50 dB, and an imaging depth of 8 cm for high line density in all studies. The focal zone was positioned at the center of the left ventricle, and gain controls were optimized for individual dogs and kept constant throughout the protocol.

Experimental Protocol
After a period of stabilization, baseline MCE and recordings of hemodynamics were performed. Thirty minutes after the injection of contrast for baseline MCE, the proximal left anterior descending coronary artery was occluded by an atraumatic vascular clamp. Occlusion was maintained for 3 hours to produce myocardial damage, after which the clamp was removed and reperfusion was implemented. MCE was repeated after 3 hours of reperfusion.

On completion of the final MCE, 2 needles were inserted into the heart so as to be observed in the echo images. These needles enabled us to excise and examine cross-sectional pathological specimens from the same level of the ventricle as the echo image. The dog was then euthanized by an overdose of pentobarbital, and the heart was dissected for tissue analysis.

Analysis of Contrast Echocardiograms
For each MCE, end-expiratory, end-diastolic video frames acquired before and 90 seconds after contrast injection were digitized by a customized system with 640×480 pixel resolution with 256 GLs. Captured images were transferred to the software package NIH Image Ver.1.5.9 for further analysis. As shown in Figure 1, intensity from an infarcted region on the anterior wall (squares) and that from a control region on the posterior interventricular septum (circles) reached maximal values by 60 to 70 seconds after injection and plateaued for 2 minutes. Thus, myocardial intensity was essentially constant, and left ventricular cavity contrast (triangles) had decreased sufficiently to eliminate significant posterior wall attenuation at 90 seconds after injection. Therefore, frames obtained 90 seconds after contrast injection were chosen for analysis. Because visual examination revealed that contrast opacification cleared uniformly from myocardial segments in nearly all animals, no specific measurements of disappearance rate were derived.

Segmentation of the myocardial wall was performed as displayed in Figure 2. First, short-axis images were divided into sectors of 15° to 30° arcs based on readily identifiable anatomic landmarks. Empirical segmentation was chosen rather than sectoring at a fixed angle to use anatomic landmarks such as trabeculae and papillary muscles to accurately match similar echocardiographic and histopathological segments. Each sector was then divided into epicar-
dial and endocardial halves. Segments of the lateral wall affected by dropout were excluded from analysis.

First, to evaluate the contrast opacification in each segment, precontrast mean pixel intensity (MPI) was subtracted from that at 90 seconds to derive background-subtracted MPI (ΔMPI). Subsequently, to quantify the heterogeneity of the contrast opacification pattern, we analyzed the echo texture on the basis of GL difference statistics.9-11 The co-occurrence matrix was constructed for each individual pixel in the segment with reference to surrounding pixels within a 16-pixel distance to derive a value for the statistical parameter entropy. Then, the average (ENTave) and maximum (ENTmax) of entropies in each segment were computed (see Appendix).

Histopathological Evaluation
With the guidance of the needles as markers, a left ventricular short-axis slice 6 to 8 mm thick was cut out at the same level at which echocardiograms were recorded. To determine infarct area, each slice was incubated in 2% triphenyltetrazolium chloride (TTC) at 37°C for 15 minutes13 and imaged on videotape, and the specimen was then fixed in 10% formalin for 48 hours for histological analysis. The images were digitized and carefully segmented, with the same landmarks used as for the echocardiogram. For each segment, both the total area and the area of the unstained region were planimetered by use of the NIH Image program, and infarct fraction (IF) was determined as the percent unstained area of the total segmental area.

Two dogs died of lethal arrhythmia due to ischemia and were excluded from analysis. Mean infarct size by TTC was 23.8±1.3% (5.3% to 37.9%) of the total myocardial area. Variations in contrast parameters were examined in an infarcted segment during the time period from 74 to 114 seconds after injection. For all of the 11 end-expiratory end-diastolic frames occurring during this period, mean±SD (SEM) and coefficient of variation were 92.7±19.0 (6.6) and 2.1% for MPI, 2.701±0.028 (0.008) and 1.0% for ENTave, and 2.811±0.034 (0.01) and 1.2% for ENTmax. Thus, these small variations suggested that overall reproducibility of the texture computation was sufficient in our study.

We were able to match and compare a total of 224 segments from 12 dogs by echocardiography and pathology. The echocardiographic and gross pathological images obtained from a representative dog are shown in Figure 3.

Classification of Segments
On the basis of TTC staining, 97 segments showed no infarction and 43 segments manifested relatively small IF (IF<30%). Because the mean IF in the small infarct segments was only 15.2% and was considered to be insignificant, these regions were combined with the normal segments. These 140 segments with no or small infarction served as the control group (Control). Significant infarction (IF>30%) was seen in 84 segments. By a definition for normal opacification of a ΔMPI greater than the lower 95% confidence limit of all segments in each dog, however, only 52 of 84 segments (62%) with significant infarction exhibited reduced contrast opacification. The infarct segments exhibiting the low-or no-reflow phenomenon by MCE composed the (−)OPAC group. A considerable number of segments with significant infarction by TTC, 32 segments, or 38%, yielded ΔMPI in the normal range and were categorized as the (+)OPAC group. Nine (75%) of the 12 dogs exhibited such infarcted segments with opacification. The prevalence of epicardial segments was 12 of 52 (23%) and 11 of 32 (34%) segments in (−)OPAC and (+)OPAC, respectively (P=NS).

Contrast Opacification and Alteration in Echo Texture
Results for contrast opacification and echo texture parameters are summarized in the Table and Figures 4 and 5. There were
no significant differences in ΔMPI among the groups at baseline. After reperfusion, although the precontrast intensity was higher in both (−)OPAC (38.0±1.8 GL) and (+)OPAC (38.2±2.7 GL) than Control (27.1±0.8 GL), (−)OPAC yielded a significantly lower ΔMPI than Control (P<0.0001), but no difference in ΔMPI was found between (+)OPAC and Control, as seen in Figure 4.

There were no significant differences in the texture parameters among the groups at baseline. After reperfusion, ENT_{ave} and ENT_{max} increased significantly from baseline only in (+)OPAC (both P<0.01) (Table). Therefore, as shown in Figure 5, at 3 hours of reperfusion, both ENT_{ave} (left) and ENT_{max} (right) were found to be significantly higher in (+)OPAC than Control (P<0.01 and P<0.001, respectively). Figure 6 demonstrates the difference in the spatial distribution patterns of GL between normal and infarcted segments from a representative study. Thus, texture parameters could differentiate infarcted but opacified segments from control segments.

### Histopathological Parameters

There was no significant difference in IF between (−)OPAC (80.9±3.1%) and (+)OPAC (73.8±3.9%, P=NS). Light microscopic photographs of hematoxylin and eosin–stained specimens taken from segments in (−)OPAC (left) and (+)OPAC (right) are shown in Figure 7. The degree of intracapillary erythrocyte stasis was higher in (−)OPAC (2.2±0.2) than (+)OPAC (1.7±0.2, P<0.05). No significant differences were observed between the 2 groups for the rest of the tissue injury findings or the total score of each finding (11.0±0.5 versus 10.6±0.6, P=NS) (Figure 8).

### Discussion

Detection of successful reperfusion of an occluded coronary artery is one of the most frequently proposed clinical applications of intravenous MCE. Previous studies using direct intracoronary contrast injection have shown that failure to produce myocardial opacification by MCE nearly always indicates necrotic tissue.1–7 Although dense enhancement generally indicates normal perfusion and viable myocardium, contractile performance failed to improve in >50% of the segments that manifested normal opacification after reperfusion therapy.5,6 The results of the present study document that necrotic myocardium can be opacified by an intravenous contrast agent early after coronary reperfusion. In such cases, conventional assessment of contrast opacification by measurement of MPI is similar in infarcted segments and those that are viable. Echo texture analysis, however, which quantifies the heterogeneity of 2D GL distribution,9–11 yielded an altered pattern of contrast opacification in infarcted segments. Thus, these data suggested that texture characterization of the contrast opacification pattern has the potential to complement conventional intensity measurements of intravenous MCE in determining myocardial viability after reperfusion. This is the first application of echo texture analysis to quantify altered opacification pattern produced by intravenous MCE in infarcted segments after reperfusion.
Mechanism of Opacification in Infarcted Segments

Several possible mechanisms might explain the normal opacification in the histologically infarcted segments in the present study. A number of microcirculatory pathophysiological phenomena could occur in the reperfused bed alone or in combination. In conjunction with the characteristics of the contrast agent used in this study, these phenomena might allow opacification of some infarcted segments.

Microcirculatory Phenomena in Reperfused Myocardium

Flow restoration to noninfarcted areas contained in a segment, which is frequently hyperemic, could pseudonormalize the “mean” pixel intensity in the overall segment. There was no significant difference, however, in the mean IF between (+)OPAC and (-)OPAC. Moreover, we found a normal magnitude of contrast opacification in 6 of 20 segments that were entirely contained within infarcted areas (IF=100%). Therefore, contrast uptake by viable myocardium within the infarcted segments cannot fully explain the normal ΔMPI in (+)OPAC.

It has been demonstrated that infarcted segments can show hyperemia above control after reperfusion that persists for several hours before gradual flow diminution. We recently demonstrated that both MCE and microsphere measurements manifested a gradual decrease, resulting in considerable residual flow in the infarcted area at 3 hours of reperfusion. Thus, at 3 hours of reperfusion, a considerable number of infarct segments can manifest low-level residual flow. Such residual flow could deliver microbubbles into the infarcted area and explain contrast enhancement. Although the severity of tissue injury was similar for both segment groups, the degree of intracapillary erythrocyte stasis was greater for (-)OPAC. The preservation of vessel integrity despite the necrosis of myocytes may represent the mechanism by which microbubbles could produce normal contrast intensity.

Characteristics of the Contrast Agent

The accuracy of identifying a no-flow region is highly dependent on the sensitivity of the method used to detect flow. If the threshold is high, a low- or no-reflow will be readily displayed even in the presence of considerable remaining perfusion. In this regard, Ambrosio et al used thioflavin S dye, which has a relatively high threshold requirement to detect flow (0.4 mL·min⁻¹·g⁻¹), and successfully demonstrated progressive deterioration of flow resulting in no-reflow in infarcted segments at 3.5 hours after reperfusion. Conversely, MCE with intracoronary Albunex has been found to be more sensitive to detect flow and has opacified myocardial segments with only 15% of normal resting flow level (0.15 mL·min⁻¹·g⁻¹). Thus, MCE has been found to be sensitive enough to detect flow and to underestimate the amount of necrosis early after reperfusion.

Dodecafluoropentane microbubbles are known to be resistant to ultrasound energy. Therefore, even in the infarcted segments in which microbubble supply is limited or ongoing postreperfusion hemorrhage traps microbubbles in extravascular space, ultrasound exposure may not eliminate micro-

Figure 6. Results of co-occurrence matrix analysis for contrast echocardiogram at 3-hour reperfusion obtained from same study as Figure 3. Matrix from infarcted segment B exhibited a broader base and lacked high peaks seen in normal segment A.

A. Control
B. Infarct

Figure 7. Light photomicrographs (hematoxylin and eosin stain) from segments in (-)OPAC (A) and (+)OPAC (B). Presence of postischemia/reperfusion tissue damage confirmed a loss of myocardial viability in both segments. Microvessels are packed with erythrocytes in A but were more patent in B (arrows indicate capillaries). Magnification ×400.
bubbles from the imaging field. In addition, transient adherence of the microbubble to the endothelium of postcapillary venules\textsuperscript{19} might cause an accumulation of bubbles in low-flow regions to detectable levels. Other investigators, however, reported that albumin and lipid microbubbles could persist in the reperfusion area by adhering to the leukocytes, which are activated by ischemia/reperfusion stress.\textsuperscript{20} Moreover, contrast opacification in infarcted myocardium has been observed with other agents.\textsuperscript{4–6} Therefore, such behavior may not be unique to dodecafluoropentane, and our findings are most likely applicable to all microbubbles. Nevertheless, the specific behavior in posts ischemic myocardium remains to be determined for each agent.

Previous data from Grayburn et al\textsuperscript{8} obtained with a similar dodecafluoropentane microbubble demonstrated a close correlation between the size of the perfusion defect by MCE and infarct size by TTC. However, this study differed from ours in several significant ways, including occlusion and reperfusion times, dose of the agent, and nature of the analysis and measurements. These variables probably explain the differences in the 2 studies.

Altered Texture in Infarcted but Opacified Myocardium

In the present study, we used a co-occurrence matrix that characterizes the occurrence of GL combinations in pairs of spatially related pixels (see Appendix). This method has been used successfully to quantify altered myocardial ultrasonic properties in amyloidosis,\textsuperscript{10} myocardial damage caused by contusion,\textsuperscript{8} and cardiac rejection.\textsuperscript{11} Using this method, we were able to detect increased entropy values in contrast-enhanced infarcted segments. Ischemia and subsequent reperfusion injury, including edema, hemorrhage, and loss of vascular integrity, cause derangement of microvascular structure and distribution, which should lead to spatial heterogeneity of microbubble distribution, resulting in an altered texture of the contrast-enhanced image.

Limitations of This Study

The acoustic properties of tissue were translated into echo texture by the ultrasound instrument. Therefore, the reproducibility of texture parameters depends on instrument settings and performance. The ultrasound data in this study were enhanced by the use of a broadband frequency transducer and a strengthened beam former.

Because only a single frame after bolus injection of microbubbles was analyzed, the temporal change in contrast opacification could have influenced reproducibility of the texture parameters calculated. By virtue of its time course of opacification, however, QW7437 allowed a long period for image acquisition, during which opacification was essentially constant (Figure 1), resulting in small variability of the calculations.

It was very important to match the segments on baseline and postreperfusion echo images with those of histopathological specimens. The geometry of the left ventricle can change after ischemia-reperfusion, however, and the shape of the ventricle and ventricular wall with regional infarction under systemic pressure differs from that of the sliced histopathological specimen, which is free of pressure. We found in a pilot study that segmentation using anatomic structure allowed more accurate matching between control and postreperfusion images and between echo images and histopathological specimens and accordingly used this method to minimize error.

In the present study, we reported only entropy derived from the co-occurrence matrix of GL pairs. Several measures are derivable from this analysis, such as angular second moment and second difference moment.\textsuperscript{9,10} Furthermore, there are several approaches to echo texture analysis, such as GL run-length statistics and edge count.\textsuperscript{9,10} Further investigation is necessary to determine whether other parameters obtainable from co-occurrence matrix or other statistics may yield better texture differentiation of injured and normal tissue after reperfusion. Similarly, only 1 contrast agent was validated in this study. Finally, regions with a small infarct zone were included as controls and in fact had entropy values similar to normal values. Thus, texture analysis may yield abnormal values only for segments with relatively large infarct areas.

Clinical Implications

In the clinical setting, only data after reperfusion are likely to be available in patients with acute myocardial infarction. This study suggests that MCE images obtained 3 hours after reperfusion therapy may allow the assessment of myocardial viability by revealing pseudonormalized contrast opacification in irreversibly injured segments, although postinfarct viability may be influenced by further reperfusion injury or no-reflow phenomenon. With the use of intravenous contrast agents, such images may be easily obtainable outside of the catheterization laboratory in patients who have undergone revascularization. In addition, this method has the potential to identify infarcted segments relatively early after reperfusion without adjunctive vasodilator stress. In this regard, our method is not affected by residual hyperemia but uses it to deliver microbubbles into infarcted segments.

Conclusions

We have demonstrated that infarcted myocardial segments can be opacified by an intravenous myocardial contrast agent early after reperfusion. Texture analysis of contrast-enhanced images revealed an inhomogeneous opacification pattern in infarcted segments. Combination of intensity measurements and texture analysis of MCE may be of potential value in...
determination of myocardial viability after coronary reperfusion.

Appendix

We used GL difference statistics in the form of co-occurrence matrix to measure the heterogeneity of GL distribution in an echocardiogram. On the basis of a pilot study, the gray values were converted into 64 shades of gray after digitization. Then echo texture parameters, ENTave, and ENTmax were computed as demonstrated in Figure 9. First, we placed a given square region (33 × 33 pixels) as a “sample window” that had a pixel (X) at the center (Figure 9A). Within this window, we paired every pixel with those separated by a “sample window” that had a pixel (X) at the center (Figure 9A). We repeated this procedure by scrolling the sample window within the segment so that each pixel had an entropy value. Subsequently, the shape of the histogram was quantified by calculating the entropy as

\[ \text{entropy} = - \sum_{g_i=0}^{63} \sum_{g_j=0}^{63} P(g_i, g_j) \ln[P(g_i, g_j)]. \]

Then the entropy value thus obtained from a sample window was placed on the pixel at its center (pixel X in Figure 9A).

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References


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