Contrast Echocardiography Can Assess Risk Area and Infarct Size During Coronary Occlusion and Reperfusion: Experimental Validation

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OBJECTIVES
We sought to validate the ability of real-time myocardial contrast echocardiography (MCE) measures of opacification defect and contrast refilling parameters to estimate risk area (RA) and infarct area (IA) during coronary occlusion and reperfusion.

BACKGROUND
No data exist establishing the accuracy of MCE in determining RA and IA size. We hypothesized that in the setting of coronary occlusion, MCE should identify RA as a perfusion defect early after bubble destruction, collateral flow to viable myocardium as opacification late during refilling and IA as absent opacification.

METHODS
Three hours of coronary occlusion and reperfusion were each produced in 11 dogs in which real-time MCE was performed during intravenous infusion of Sonovue (Bracco). Real-time contrast echocardiography was performed at baseline, during occlusion and reperfusion. Early (BEGIN) and end (END) images from a FLASH refilling sequence were acquired, as well as late refilling images (LATE) 1 min after FLASH. Real-time contrast echocardiography defect size and quantitative refilling parameters were compared with RA and IA determined by tissue staining.

RESULTS
During occlusion, defect size varied with refilling time; defects from BEGIN images correlated best to RA and those from LATE images to IA. Refilling parameters, but not LATE peak intensity, did not predict the IA size during occlusion. During reperfusion, defects from BEGIN images were well correlated to RA and END images to IA, whereas peak plateau intensity and refilling slope parameters predicted IA size.

CONCLUSIONS
Real-time contrast echocardiography defect size varies throughout microbubble refilling. Appropriately selected defect sizes and refilling parameters provide estimates of RA and IA during coronary occlusion and reperfusion. (J Am Coll Cardiol 2002;39:1546–54) © 2002 by the American College of Cardiology Foundation
an ultrasonic transit-time flow probe (2.0 to 2.5 mm) was positioned proximal to the occluder and connected to a flow-meter (model T201; Transonic System Inc., Ithaca, New York). Three 7F catheters were placed: left femoral artery for withdrawal of reference samples for blood flow measurements, right femoral artery to record arterial pressure and left femoral vein to infuse drugs and fluids. A catheter (Swan-Ganz) monitored pulmonary artery pressures and measured wedge pressure.

Myocardial contrast echocardiography was performed with a real-time ultrasound system (HDI5000, ATL, Bothell, Washington) capable of low energy (0.13 to 0.15 mechanical index) MCE. Short-axis midpapillary muscle images were obtained with the transducer fixed on a saline-filled latex bag positioned on the left ventricular anterior wall. Instrument settings were optimized and held constant for each dog. Sonovue (Bracco Inc., Milano, Italia) was continuously infused at a rate of 30 ml/h by a gently agitated infusion pump. Two successive real-time sequences were recorded: the first was a FLASH sequence, consisting of 15 cardiac cycles (maximum buffer memory allowed) initiated by three high energy (1.0 MI) transmission images, which produced bubble destruction and enabled visualization of subsequent refilling. The second (LATE) sequence was also acquired during real-time mode and consisted of five cardiac cycles recorded 1 and 2 min after FLASH transmission. From these sequences, end-systolic images were carefully selected for subsequent quantitative analysis to avoid motion artifacts.

**Image analysis.** Sequences were acquired during occlusion and reperfusion and were compared to baseline to identify perfusion abnormalities. After pictures were transferred to commercial image analysis software, the borders of perfusion defects were visually identified as the largest area with clearly diminished opacification and the area measured by digital planimetry by two observers blind to conditions. The images from four real-time phases were specifically analyzed and compared with each other: 1) the initial two to four cardiac cycles following FLASH transmission (BEGIN), 2) the final two to four refilling cardiac cycles at the end of the 15-cycle FLASH refilling sequence (END) and five cardiac cycles at 1 and 2 min after the FLASH transmission (LATE) (Fig. 1). Defect size was expressed as the percent of the total planed myocardial area from the same image.

**Refilling parameter calculation.** The raw data were analyzed using HDI Lab (ATL) software. Two equal-sized regions of interest were manually traced in the mid-wall myocardium: one including the defect from BEGIN and one in the center of a comparable adjacent area without visualized defect (control area, CA). The region of interest was drawn to encompass nearly the entire BEGIN defect with the exclusion of high intensity endocardial and epicardial borders. Myocardial contrast echocardiography intensity throughout the FLASH imaging sequence was plotted against time and fitted to the exponential function \( y = A(1 - e^{-bt}) + c \), where \( A \) was the plateau signal intensity reflecting the cross-sectional blood volume, \( b \) the rate of rise to the plateau reflecting myocardial blood velocity and \( c \) the intercept at the origin (2). Consequently, the product of \( A \) and \( b \) reflected the volume of MBF. From the late sequences, we averaged the peak signal intensity from five end-systolic pictures.

**MBF measurement and dye analysis.** Myocardial blood flow was measured by standard techniques. Briefly, fluorescent microspheres were injected into the left atrium while reference blood samples were withdrawn from the femoral artery. At the end of the experiment, the coronary artery was reoccluded, blue dye was injected in the left atrium and the RA myocardium was delineated as that without blue stain.

**Figure 1.** Diagram summarizing FLASH and LATE sequence protocol performed during occlusion and reperfusion. MCE = myocardial contrast echocardiography.
Following the insertion of needles for identification, the myocardial cross-section corresponding to the echo short-axis image was acquired and divided in two. One-half was incubated in a 2% solution of 2,3,5-triphenyltetrazolium chloride (TTC) for 20 min at 37°C, and the infarcted myocardium was identified as the region that failed to demonstrate brisk red staining. This slice was then photographed in color and the outlines of the entire cross-section, the RA, and the infarct zone were measured by digital planimetry. The second slice was cut into 10 equal-sized wedge-shaped transmural samples for MBF analysis using a flow cytometer to count the entrapped microspheres.

**Experimental protocol.** Hemodynamics, MBF, flowmeter measurements and real-time MCE were acquired at baseline for each animal. Coronary occlusion was then performed for 3 h. For the initial four experiments, measurements were obtained at 90 min of occlusion and just before reperfusion at 180 min. Because these values were similar, all subsequent measurements obtained and reported were performed at 90 min occlusion. Reperfusion was then implemented and measurements repeated at 30, 60, 120 and 180 min following reflow.

**Statistical methods.** Data were expressed as mean ± SD. Comparison of hemodynamics, MBF and MCE data was performed using repeated-measures analysis of variance and Student t test. Defect sizes by MCE were correlated with those by blue dye and TTC. A value of p < 0.05 (two-sided) was considered significant except for multiple comparisons concerning A and b values; these two parameters were assessed five times with baseline, so we applied the Bonferroni's correction to the threshold of significance. Consequently, the significance was set to 0.01. Reproducibility of measurements was assessed by analysis of variance.

**RESULTS**

The protocol was successfully performed in all 11 dogs. Two dogs had ventricular fibrillation during occlusion, which required external electric shock, but hemodynamics were subsequently stabilized afterward. High-quality contrast images with minimum artifacts were obtained during baseline, occlusion and reperfusion, and successful FLASH imaging was carried out, which produced profound microbubble destruction. Hemodynamic data for the entire study group are presented in Table 1. The mean RA of the study population was 32 ± 8% and the mean infarct size was 20 ± 13% of the left ventricle. At each time point during occlusion and reperfusion, MCE parameters (A and b) correlated with flow measurements obtained from microspheres (RA/CA MCE vs. MBF: y = 1.92x − 0.35, r = 0.66, p < 0.05). Myocardial contrast echocardiography consistently underestimated IA at all time points.

**Assessment of risk and IAs during occlusion.** Analysis of FLASH sequences acquired during occlusion demonstrated a clear area of decreased opacification in the perfusion bed of the occluded coronary (Fig. 2). The defect size was similar throughout the FLASH sequence (BEGIN: 35 ± 8%, END: 32 ± 11%, p = ns). The tendency for defect size to decrease during the sequence may have been related to more complete filling of the border zone at the end of the sequence. Alternatively, signal intensity in the CA was increased from BEGIN to END (3.4 ± 1.1 dB vs. 6.9 ± 1.8 dB, p < 0.05) yielding better visualization and definition of defects in the final cycles of the sequence. As opposed to the FLASH sequence, opacification defects from LATE images (1 min after FLASH sequence) were significantly smaller than either BEGIN or END (LATE: 18 ± 11%, p < 0.001) (Fig. 2), indicating that 15 cardiac cycles were insufficient to achieve complete RA perfusion. However, no significant difference was observed between LATE at 1 and 2 min, either in terms of defect measurements (18 ± 11% vs. 20 ± 9%, p = ns) or signal intensity (3.29 ± 1.72 dB vs. 3.38 ± 1.97 dB, p = ns), demonstrating that during occlusion steady-state MCE reperfusion in the RA was reached by 1 min.

Comparison with postmortem analysis established that both BEGIN and END defect size and blue dye RA sizes were closely correlated (y = 0.80x + 0.09, r = 0.84, and y = 0.75x + 0.05, r = 0.79). However, both BEGIN and END defect sizes correlated less closely with IA size by TTC (y = 0.34x + 0.13, r = 0.61, and y = 0.40x + 0.26, r = 0.69). Conversely, the correlation between LATE defects and IA sizes was closer than the correlation between LATE defect and RA sizes (y = 0.76x + 0.03, r = 0.86 for IA vs. y = 0.90x − 0.10, r = 0.61 for RA) (Fig. 3). Thus, during occlusion FLASH and LATE sequences differed in the significance of defect sizes. FLASH sequence defect size more closely reflected RA, whereas LATE sequence defect size provided a more accurate assessment of the IA.

**Measurement of MCE signal during occlusion (Fig. 4).** Within the RA, peak plateau intensity (A), refilling slope (b) and product A × b from the FLASH sequence were 5.88 ± 1.21 dB, 0.28 ± 0.12 s⁻¹ and 1.64 ± 1.10 dB.s⁻¹ at baseline, and decreased during occlusion to 1.34 ± 0.86 dB, 0.05 ± 0.03 s⁻¹ and 0.067 ± 0.068 dB.s⁻¹, respectively.

**Table 1. Hemodynamic Data**

<table>
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<th>Baseline</th>
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<th>R30</th>
<th>R60</th>
<th>R120</th>
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<td>HR (beats/min)</td>
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<td>123 ± 26</td>
<td>123 ± 17</td>
<td>122 ± 24</td>
<td>114 ± 18*</td>
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<tr>
<td>DBP (mm Hg)</td>
<td>106 ± 14</td>
<td>108 ± 17</td>
<td>95 ± 20*</td>
<td>94 ± 11*</td>
<td>94 ± 16*</td>
<td>98 ± 11*</td>
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<tr>
<td>WP (mm Hg)</td>
<td>10.8 ± 1.1</td>
<td>13.4 ± 4*</td>
<td>14.8 ± 3*</td>
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<td>14.4 ± 3*</td>
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<tr>
<td>CF (nl/min)</td>
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<td>0 ± 0*</td>
<td>16 ± 5</td>
<td>17 ± 7</td>
<td>16 ± 9</td>
<td>12 ± 5*</td>
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CF = coronary flow; DBP = diastolic blood pressure; HR = heart rate; SBP = systolic blood pressure; WP = wedge pressure.
During occlusion, the peak intensity plateau (A) value measured from LATE was significantly higher than from the FLASH sequence (3.29 ± 1.72 dB vs. 1.34 ± 0.86 dB, p < 0.005). Furthermore, the correlation between A from LATE and IA size was significantly closer than that with A, b and product A × b from the FLASH sequence (y = −10.7x + 5.4, r = 0.81, vs. y = −2.51x + 1.8, r = 0.37, y = −0.23x + 0.12, r = 0.36 and y = −0.42x + 0.16, r = 0.51, respectively, p < 0.05) (Fig. 4). Thus, peak plateau intensity from LATE, which is assumed to be due to collateral flow, showed a good correlation to the final IA, confirming data from defect size measurements.

Assessment of reperfused and IAs during reperfusion. Following reperfusion, opacification defects continued to be observed in 10 of 11 animals at END, although the defect in one animal was so small as to be nearly immeasurable. As with occlusion, we compared images from FLASH and LATE sequences during reperfusion. As opposed to occlusion, during reperfusion no difference was observed between END and LATE in either the size or signal intensity of the opacification defects (Fig. 2). For example, at the 60-min reperfusion measurement, defect sizes were 28 ± 9% from END and 27 ± 7% from LATE 1 min (p = ns). Similarly, signal intensities were 4.7 ± 1.9 and 4.9 ± 1.4 dB in reperfused area with END and LATE respectively (p = ns). Conversely, comparison clearly revealed marked differences in BEGIN and END defect measurements during reperfusion. Mean defect size was 28 ± 14%, 28 ± 9%, 31 ± 8% and 31 ± 8% at 30, 60, 120 and 180 min of reperfusion at BEGIN and 14 ± 13%, 13 ± 14%, 16 ± 13% and 17 ± 12% at END, all p < 0.01. No significant difference was observed between either mean defect size from BEGIN and reperfused area size or mean defect size from END and IA size (for example, at 180 min of reperfusion: BEGIN 31 ± 8% vs. RA 32 ± 8%, p = ns and BEGIN 31 ± 8% vs. IA 20 ± 13%, p < 0.05, and END 17 ± 12% vs. RA 32 ± 8%, p < 0.05, and END 17 ± 12% vs. IA 20 ± 13%, p = ns). Regression analysis performed between BEGIN and RA and between END and IA yielded correlation coefficients during reperfusion, as shown in Figure 5. BEGIN correlated closely with RA at all time points, whereas END correlated closely with IA. The correlation was weakest...
between BEGIN and RA at 30 min of reperfusion ($r = 0.34$) and strongest between END and IA at 180 min ($r = 0.93$).

**Quantitative MCE parameters during reperfusion (Fig. 6).** A and b parameters from the FLASH sequence were decreased compared to baseline and control during reperfusion, with statistically significant reductions observed at 120 and 180 min for the A parameter and at all reperfusion stages for the b parameter (Fig. 6). In the subgroup of three animals with large infarctions of at least 80% of the RA, we observed a marked decrease of A and b parameters at 30 min of reperfusion, which did not subsequently vary with additional reperfusion time (2.7 ± 1.4 dB at 30 min vs. 2.6 ± 0.7 dB at 180 min for A parameter, 0.12 ± 0.01 s⁻¹ vs. 0.11 ± 0.02 s⁻¹ for b parameter). Thus, 50% reductions of A and b in the RA during reperfusion were associated with a large final IA of 80% RA.

Finally, regression analysis of histologic IA sizes with A and b parameters calculated at 180 min of reperfusion demonstrated good correlation for both parameters (A parameter: $y = -8.67x + 5.94$, $r = 0.75$, and b parameter: $y = -0.32x + 0.23$, $r = 0.71$). These data demonstrate the capability of FLASH sequence parameters to predict IA size in the initial hours after reperfusion.

**Reproducibility.** Defect measurements and quantitative parameters were evaluated by two observers for interobserver variability. For all defect measurements, the variability expressed by the relative mean error was 8.2% during occlusion, whereas it was 12.4% during reperfusion. Similar results were observed for quantitative parameters from FLASH and LATE sequences during occlusion (FLASH sequence: A parameter 10.5%, b parameter 7.2%; LATE sequence: A parameter 8.3%) and reperfusion (FLASH sequence: A parameter 12.7%, b parameter 9.9%; LATE sequence: A parameter 8.1%).

**DISCUSSION**

Although several studies have demonstrated the capacity of low-energy real-time MCE to detect coronary stenosis (3,5), no data exist regarding the ability of this technique to assess acute coronary occlusion/reperfusion. We postulated that the extent and time course of microbubble refilling would distinguish normally perfused, collateral-dependent and nonperfused myocardium, and therefore differentiate RA from infarct zone associated with total coronary obstruction and reflow. The major findings of our study are as follows: 1) the size of an opacification defect varies with the time period available for microbubble refilling; 2) during occlusion, 15-cardiac-cycle FLASH sequence imaging yields a larger defect size, which correlates more closely with RA than IA, whereas imaging delayed for at least 1 min yields a smaller area of abnormal opacification that correlates more closely with IA than RA; 3) neither b nor A parameters from the FLASH sequence predict infarct size during occlusion; 4) during reperfusion, opacification defect size from a FLASH sequence can predict both RA (from images of initial cardiac cycles) and IA (from images at the end of the sequence); 5) both A and b parameters are decreased in RA myocardium compared to baseline postreperfusion, with the decrease in b occurring earlier than A; 6) 50% decreases in A and b during early reperfusion occur when infarct size is above 80% of RA; therefore, marked reduction of A and b correspond to large IA; 7) the ability of A and b parameters to predict IA after reperfusion improved over time and was best at 180 min. These data provide the basis for the clinical application of MCE to assess RA and IA during acute coronary occlusion/reperfusion in patients.

**Risk and IA assessment during occlusion.** Our major finding was that MCE was able to quantitatively assess both RA and IA during occlusion, but not from FLASH sequences alone. Thus, in the absence of antegrade flow, perfusion defects in FLASH sequence images accurately delineated RA. However, RA opacification increased progressively for 1 min after microbubble destruction, and 15 cardiac cycles were insufficient to detect the complete opacification of this region. Therefore, a smaller defect was visualized in LATE images and correlated more closely with infarct size. These data indicate that MCE can detect slow
collateral flow perfusing the RA and preventing the evolution of RA ischemia into infarction. Although previous studies have shown a high correlation between the size of MCE defects and RA (6,7), analysis of MCE refilling images acquired early and late after FLASH bubble destruction is the only technique thus far shown to provide information regarding both RA and IA during coronary occlusion.

**Risk and IA assessment during reperfusion.** Based on the same mechanism of microbubble refilling, MCE analysis also enabled assessment of both RA and IA during reperfusion. Risk area was again delineated by absent opacification in images at BEGIN, whereas IA was now depicted by perfusion defects in images at the END as well as those visualized at 1 to 2 min of refilling. Thus, in the case of reperfusion, FLASH sequences alone can be used to assess RA and IA, indicating that blood flow velocities are generally homogeneous and that microbubbles refill the myocardium within the 15 cardiac cycles following restoration of flow. Moreover, as will be discussed, MBF abnormalities were identified by quantitative analysis of A and b parameters from refilling curves during reperfusion.

The correlation with IA observed during reperfusion was similar to that reported by Villanueva et al. (8) using intracoronary contrast with adenosine. In the prior study, correlations without adenosine were less than those observed in our study. Several factors may explain these differences: only fundamental grayscale imaging was available to Villanueva and the concentration of myocardial microbubbles with intravenous injection of contrast agent is lower than with intracoronary injection. However, MCE still underestimated RA and IA areas in our study (Figs. 2 and 5), confirming the discrepancy between capillary integrity and myocardial cell necrosis, which can be reduced by using pharmacologically induced hyperemia. Moreover, real-time MCE with power pulse inversion is still subject to the “blooming” phenomenon.

Prior studies by us (9) and others (10) have found that myocardium that is infarcted histologically or noncontractile after revascularization may exhibit opacification by MCE. Similar findings were observed in two of 11 experiments. The greater frequency with which infarcted myocardium in this study was observed to exhibit a perfusion defect by MCE is again likely due to the low-energy real-time
imaging technique, intravenous injection and nature of the microbubble agent employed, and may have implications for the clinical setting.

Finally, the long interval required for RA opacification seemed inconsistent with the velocity of collateral flow, suggesting that some microbubble destruction may have occurred. However, the discrepancy between defects acquired during occlusion and reperfusion (smaller defect with the FLASH sequence during reperfusion) is most likely due to an increase of collateral flow during reperfusion combined with the addition of anterograde flow, which improved microvascular flow to the viable areas of the perfusion bed. This hypothesis is supported by the results of Ambrioso et al. (11), who observed an increase of regional MBF in both RA and CA compared to baseline and ischemia stages.

Quantitative parameters from FLASH sequence. In the current study we utilized microbubble destruction/refilling curves derived from low-energy real-time MCE. Electrocardiogram-gated approaches to quantify MCE refilling have also been developed and are available for clinical application. Coggins et al. (12) recently demonstrated that ECG-gated acquisition was able to analyze both RA and IA by varying pulsing intervals. Recent studies in our laboratory have also shown that the refilling parameters derived from real-time and ECG-gated imaging sequences are similar. Therefore, the MCE approach to assessing RA and infarct size during coronary occlusion and reperfusion should be applicable to both real-time and triggered MCE approaches.

We had hypothesized that quantitative parameters from MCE refilling curves obtained during coronary occlusion would predict ultimate infarct size. This clearly was not the case. Rather, the contrast intensity recorded from the perfusion bed during coronary artery occlusion appears insufficient to accurately yield quantitative refilling parameters. However, quantitative measurements were able to distinguish the occluded area from the reperfused area both with A and b parameters (Fig. 6).

Analysis of our data indicated that quantitative parameters derived from refilling curves during myocardial reperfusion could distinguish reperfused myocardium from that which was unaffected by reduced coronary flow. Thus, following reperfusion both A and b parameters were decreased in the RA as opposed to an adjacent CA. Nevertheless, TTC staining indicated that, in the absence of profound (50% or greater) reduction in quantitative parameters, the myocardium exhibits viability. Therefore, assessment of refilling MCE parameters from postreperfusion myocardium may be of value in distinguishing RA from normally perfused myocardium, and may be capable of indicating areas of infarcted muscle by virtue of profound reduction in magnitude.

The findings of this study indicated that quantitative parameters of MCE refilling correlated best with ultimate evidence of infarction when obtained 60 to 180 min postreperfusion. These data suggest that, if this approach is to be utilized clinically, assessment of infarction versus viability by MCE should be delayed for at least 1 h following the reestablishment of flow. Nevertheless, even these data may be of value in patients initially undergoing echocardiography some time after reperfusion therapy. It

Figure 5. Correlations between risk area (RA) and infarct area (IA) sizes obtained by postmortem dye methods and FLASH sequence defects (beginning of the sequence, BEGIN, and end of the sequence, END, respectively) at four time points of reperfusion. MCE = myocardial contrast echocardiography.
was somewhat surprising that a quantitative measurement of the time course of contrast refilling derived from only a portion of the RA should be predictive of the ultimate percent of RA undergoing infarction. Rather, one would have predicted that refilling parameters should be specific to the area of myocardium from which they were derived. It is likely that the predictive correlation between refilling parameters and the ratio of infarct size to RA observed in this study may be due to an attempt to include intensity measurements from as large a portion of the RA as possible. **Clinical implications.** Our study provides a basis of the application of MCE to myocardial infarction. We have shown it is possible to both visualize and quantitatively assess risk and IAs during coronary occlusion. Furthermore, MCE can assess the efficacy of reperfusion treatment, which may guide subsequent patient management, but is limited by requiring 180 min to be most accurate. This experimental study has also delineated cautions that must be observed when using MCE. Specifically, the opacification defect visualized is determined in large measure by the refilling time at which images are obtained. It is therefore very important to acquire both FLASH and LATE sequence data so as not to miss any perfusion information. However, this temporal variation provides the basis of distinguishing RA from IA.

**REFERENCES**


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