Comparison of Real-Time and Intermittent Triggered Myocardial Contrast Echocardiography for Quantification of Coronary Stenosis Severity and Transmural Perfusion Gradient

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Background—Both intermittent triggered and real-time myocardial contrast echocardiography (MCE) have been proposed to detect impaired myocardial perfusion. We compared the ability of these 2 methods to quantify altered myocardial blood flow (MBF) and transmural distribution of MBF produced by graded coronary stenoses.

Methods and Results—In 8 open-chest dogs, we created 4 graded left anterior descending coronary artery (LAD) stenoses: 3 levels of reduced adenosine hyperemia (non–flow-limiting at rest) and 1 grade of flow-limiting at rest. Real-time MCE was performed with SonoVue infusion using low-energy power pulse inversion (ATL) imaging, whereas ECG-gated intermittent triggered imaging used high energy at pulsing intervals from 1:1 to 1:10. LAD signal intensity (SI) was plotted versus time by real-time MCE and versus pulsing intervals by triggered MCE and was fitted to a 1-exponential function to obtain plateau SI (A) and the rate of SI rise (b). Visual detection of decreased opacification was equivalent by triggered and real-time MCE. Fluorescent microsphere–derived MBF ratio in LAD/left circumflex artery beds demonstrated close correlation with both real-time imaging (b, \( r = 0.79 \); A×b, \( r = 0.81 \)) and triggered imaging (b, \( r = 0.78 \); A×b, \( r = 0.80 \)). The endocardial/epicardial ratio of MBF in the LAD bed demonstrated closer correlation with the endocardial/epicardial ratios of b ( \( r = 0.71 \)) and A×b ( \( r = 0.67 \)) obtained by real-time than triggered imaging (b, \( r = 0.42 \); A×b, \( r = 0.52 \)).

Conclusions—Real-time and triggered MCE are equivalent in their ability to identify coronary stenosis and quantify altered MBF. (Circulation. 2001;104:1550-1556.)

Key Words: echocardiography • perfusion • blood flow

The ability of myocardial contrast echocardiography (MCE) using ECG-gated intermittent triggered imaging to quantify myocardial perfusion has been established.1-3 Wei et al3 applied progressively prolonged intervals between ECG-gated imaging pulses and demonstrated that signal intensity (SI) increased over time until a peak plateau value was reached at which refilling of the imaging field between pulses was complete. They fitted the time-intensity data to an exponential equation as \( y = A(1-e^{-\beta t}) \), where A was peak plateau intensity and b was the rate of signal increase, and demonstrated that both b and the product of A and b could be correlated with myocardial blood flow (MBF).

Recent technological advances have enabled myocardial opacification by MCE to be achieved during low-energy real-time imaging.4-5 By this approach, high-energy ultrasound bursts can be periodically transmitted to produce bubble destruction, after which consecutive frames delineate the restoration of contrast intensity. Time-intensity plots from the resultant data can be subjected to curve-fitting and provide measures of the rate of increase and the peak level of SI. At present, no data exist comparing real-time MCE time-intensity curves with those obtained with triggered MCE with respect to the ability to identify and quantify perfusion abnormalities produced by coronary stenosis. Therefore, we compared the ability of real-time MCE versus triggered imaging to assess myocardial perfusion abnormalities produced by graded coronary stenoses and to correlate SI parameters derived from time-intensity curves with measures of regional MBF.

Methods

Animal Preparation

In 8 anesthetized mongrel dogs, femoral artery catheters (7F) measured hemodynamics and provided blood samples. 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 (LAD) was dissected free from the surrounding...
was assessed.6 Myocardial SI was measured in gray-scale units offline computer. Color was ignored, and only myocardial luminance before and after microbubble injection at each pulsing interval were triggered MCE, end-systolic images acquired on S-VHS videotape as a measure of MBF.3 To measure myocardial SI from intermittent cross-sectional area or myocardial blood volume, b is the rate of SI every frame (every phase of cardiac cycle) versus time after FLASH digital cine loop by use of HDI Laboratory software. LAD bed SI at replenishment.

(1:10) cardiac cycles to allow incremental microbubble pulse repetition frequency of 2500 Hz were used. The interval time scanning allowed microbubble replenishment into the ultra-microbubbles to yield unopacified myocardium. Low-energy real-(FLASH) frames transmitted every 15 cardiac cycles destroyed image. Four consecutive 0.8 mechanical index fast, low-angle shot pilot experiments, a low dynamic range and pulse-repetition frequency of 2500 Hz was selected to achieve definite visible myocardial opacification. Instrument settings were constant for each experiment. Four consecutive 0.8 mechanical index fast, low-angle shot (FLASH) frames transmitted every 15 cardiac cycles destroyed microbubbles to yield unopacified myocardium. Low-energy real-time scanning allowed microbubble replenishment into the ultrasound field.

After recording of real-time MCE, ECG-gated intermittent imaging was performed with end-systolic ECG triggering by color-coded harmonic power-pulse inversion. The mechanical index was fixed at 1.2. On the basis of pilot experiments, a high dynamic range and pulse-repetition frequency of 2500 Hz were used. The interval between the ECG triggers (pulsing interval) was increased from every heartbeat (1:1) to every 2 (1:2), 4 (1:4), 6 (1:6), 8 (1:8), and 10 (1:10) cardiac cycles to allow incremental microbubble replenishment.

To measure myocardial SI from real-time MCE, frames preceding and following FLASH frames were digitally captured and analyzed offline. Myocardial SI was measured for each 15-cardiac-cycle digital cine frame loop by use of HDI Laboratory software. LAD bed SI at every frame (every phase of cardiac cycle) versus time after FLASH was fitted to an exponential function: \( y = A(1 - e^{-bt}) \), where y is SI at any given time, A is the plateau SI that reflects microvascular cross-sectional area or myocardial blood volume, b is the rate of SI rise (slope of the curve) that reflects myocardial microbubble velocity, and t is time after FLASH. The product of A and b provides a measure of MFB.3 To measure myocardial SI from intermittent triggered MCE, end-systolic images acquired on S-VHS videotape before and after microbubble injection at each pulsing interval were transferred to an offline Macintosh computer and aligned for background subtraction. Images by color-coded power-pulse inversion were converted to gray scale by use of NIH Image 1.62 on the offline computer. Color was ignored, and only myocardial luminance was assessed.6 Myocardial SI was measured in gray-scale units ranging from 0 to 255. To determine whether analog videointensity measurements yielded results similar to digital data for triggered imaging, we compared both methods (analog versus digital) in 4 of the 8 dogs. Because no differences were observed and the correlations between MCE and MFB ratios were similar, we performed videointensity measurements for SI analysis for triggered imaging in all 8 dogs. For analysis of both imaging modalities, the LAD perfusion territory was identified as the transmural area of absent opacification after contrast injection during coronary occlusion. The area of absent opacification during coronary occlusion was determined at a pulsing interval of 1:1 for triggered imaging and at the 14th cardiac cycle after FLASH for real-time imaging. Transmural regions of interest were selected that encompassed the LAD perfusion territory and adjacent nonattenuated area of the left circumflex coronary artery (LCx). The ratio of LAD/LCx values was then used to normalize intensity values and was subjected to linear regression analysis compared with MFB. In addition, the LAD bed was biseected, and the ratio was calculated for the endocardial and epicardial halves.

MBF Measurement
MBF was measured by injection of fluorescent microspheres (Molecular Probes) into the left atrium during blood withdrawal from the femoral artery. After the animal was euthanized, the heart was sliced into cross-sectional segments, and the slice corresponding to the short-axis image was cut into 12 wedge-shaped transmural tissue pieces, each of which was divided into endocardial and epicardial segments. The tissue and the arterial reference sample were processed to count the microspheres. MFB to each endocardial and epicardial segment was calculated from the equation \( Q_m = (C_m \cdot Q_r)/C_e \), where \( Q_m \) is the blood flow to the myocardial segment (mL/min), \( C_m \) is tissue count, \( Q_r \) is rate of arterial sample withdrawal (mL/min), and \( C_e \) is arterial reference sample count.7 Transmural MBF (mL · min⁻¹ · g⁻¹) to 12 wedge-shaped pieces was calculated as the quotient of the summed flows to the individual segments within that piece over the combined MBF. MFB to the LAD and LCx beds defined by monastral blue dye (Sigma Chemical Co) injection was then calculated by averaging the transmural MBF in the pieces from each bed respectively. The LAD/LCx ratio of transmural MBF and the ratio of endocardial/epicardial MBF in the LAD bed were calculated.

Experimental Protocol
After baseline MCE and MFB data had been acquired, adenosine was infused intravenously at 140 \( \mu \)g · kg⁻¹ · min⁻¹. A high hyperemia was reached, MCE and MFB data were repeated. Thereafter, guided by flow probe, the LAD was progressively compressed with a screw occluder to produce 3 levels of non–flow-limiting stenosis (NFLS), which reduced the increase in LAD flow produced by hyperemia (ie, coronary flow reserve) by ~30% (mild), 60% (moderate), and 90% (severe). MCE and MBF measurements were performed at each NFLS. After the 3 levels of NFLS, the adenosine infusion was stopped and LAD flow was allowed to decrease to baseline value in all dogs. Thereafter, a flow-limiting stenosis (FLS) was created by reducing LAD flow by 50% of the baseline value. Adenosine was infused again, and MCE and MFB data were reacquired. Finally, total LAD occlusion was produced and MCE and MBF measurements were performed. At the end of the experiment, the LAD was occluded at the occluder site, and monastral blue dye (Sigma Chemical Co) was injected into the left atrium to delineate the LAD and LCx beds. The dog was then euthanized, and the slice corresponding to the MCE imaging plane was processed for fluorescent microsphere analysis.

Statistical Analysis
Data were expressed as mean±SD. Comparisons of hemodynamics, MBF, and MCE data among all stages were performed by repeated-measures ANOVA, and Bonferroni t test was used to assess the statistical difference between multiple comparisons. Correlations between MBF and MCE data were performed by linear regression analysis. For differences, a value of \( P<0.05 \) was considered significant.

Results
Severity of Coronary Stenosis
The LAD/LCx ratio of MBF by fluorescent microspheres progressively decreased with greater levels of stenosis (Figure 1). The reduction in LAD/LCx ratio was significant at all levels except mild NFLS. The endocardial/epicardial ratio of MBF-measured fluorescent microspheres in the LAD bed was <1.0 in moderate or greater NFLS, and the reduction from without stenosis was statistically significant (\( P<0.05 \)) in severe or greater NFLS (Figure 1).
Visual Analysis of Stenosis

Figure 2 shows changes in end-systolic images during various pulsing intervals by triggered imaging with each level of stenosis in an animal that showed a perfusion defect with all grades of stenoses. All grades of stenosis showed opacification defects at a pulsing interval of 1:1. The defects disappeared or were reduced in size, however, with prolongation of pulsing intervals in all grades of stenoses. The defects persisted, however, even during long pulsing intervals at the most severe grades of stenosis.

Figure 3 shows changes in consecutive end-systolic MCE images by real-time MCE after FLASH with each level of stenosis in the same animal as presented in Figure 2. Myocardial SI by real-time imaging was not as intense as that by triggered imaging. Because microbubbles in the myocardium were destroyed by the high-energy frames, both the risk myocardium (LAD) and control (LCx) regions were unopacified at the first cardiac cycle after the FLASH with all flow states. Without stenosis, from the second cardiac cycle after FLASH, the SI of both regions increased quickly and equally because of prompt replenishment of microbubbles into the myocardium. The speed of SI rise after FLASH looked to be similar in risk and control areas by visual analysis in the absence of stenosis. Opacification defects were observed with all grades of stenosis, however, because of slow replenishment of microbubbles into the LAD region. All grades of stenosis showed defects during the early phase (second cardiac cycle) after FLASH. The defects disappeared or were reduced in size, however, as time after FLASH increased. With severe stenoses, the defects persisted even in the last phase (14th cardiac cycle) of sequences between 2 FLASHes. Thus, temporal changes in opacification defects after FLASH by real-time imaging were similar to those observed during various pulsing intervals by triggered imaging. Although recognition of transmural differences in the opacification defect was difficult with triggered imaging, the opacification defects were predominantly in subendocardial regions during moderate and severe NFLS and FLS with real-time imaging.

Myocardial SI in the Presence of Graded Coronary Stenosis

Figure 4, left, shows averaged values for myocardial SI plotted versus pulsing interval plots of triggered imaging at each level of stenosis from the LAD risk area in 8 dogs and the results of fitting the data to the 1-exponential function described in Methods. These curves demonstrated that the values both of the rate of SI rise (b) and of plateau SI (A) at the tail end of the curve progressively decreased with greater levels of stenosis. Figure 4, right, shows averaged values for myocardial SI versus time plots by real-time imaging in the risk area derived from curve fitting at each stage in 8 dogs. Note that the magnitude of SI by real-time imaging was smaller than that by triggered imaging. These curves, however, also demonstrated that the values both of b and of A at the tail end of the curve progressively decreased with greater levels of stenosis, similar to the curves obtained by triggered imaging.
Comparison of MCE-Derived Parameters During Graded Coronary Stenoses
Mean values for LAD/LCx ratio of A, b, and A×b at each level of stenosis are presented for triggered imaging and for real-time imaging in Figure 5. The reduction in LAD/LCx ratio of A was greater at each level of stenosis with real-time imaging than with triggered imaging, and this decrease was significant compared with normal and severe NFLS and FLS by real-time imaging, but only with FLS by triggered imaging. The LAD/LCx ratio of b and A×b progressively decreased to a similar extent with greater levels of stenosis and demonstrated a significant reduction compared with normal, even with mild NFLS, with both imaging modalities.

Correlation Between MBF and Parameters Derived From MCE
Figure 6 shows the correlation between the MCE- and the fluorescent microsphere–derived MBF ratios from the stenosed versus normal beds. The correlation of the A ratio with MBF ratio was slightly closer with real-time imaging than with triggered imaging. The correlation with MBF of b and A×b parameters was closer than that of A with both imaging modalities, and the correlation was not different between the 2 imaging modalities.

Transmural Differences of MCE-Derived Parameters in the Presence of Graded Coronary Stenosis
Figure 7 shows the endocardial/epicardial ratios of MCE-derived parameters in the LAD bed by triggered imaging (left) and real-time imaging (right). With triggered imaging, no significant alteration was observed in any parameter with any level of stenosis, even though all parameters tended to decrease with greater levels of stenosis. With real-time imaging, all parameters progressively decreased with greater levels of stenosis. Although A demonstrated significant reduction compared with normal only with FLS, b demonstrated significant reduction with severe NFLS and FLS. Moreover, A×b demonstrated significant reduction even with moderate NFLS.

Correlation Between MCE and MBF Endocardial/Epicardial Ratios
Figure 8 shows the correlation between MCE and the microsphere-derived MBF endocardial/epicardial ratios in the LAD bed. The correlation between MCE and MBF ratios was closer for b and A×b than for A with both imaging modalities. The correlation obtained by real-time imaging was slightly closer than that by triggered imaging for each parameter of MCE.
Discussion

The ability to derive parameters of myocardial perfusion from the time course of intensity return after microbubble destruction has provided an objective quantitative criterion to identify abnormal blood flow by MCE. The optimal method to acquire such measures, however, remains uncertain. Real-time low-energy MCE allows simultaneous evaluation of contractile function and provides a larger spectrum of pulsing intervals from which to calculate refilling curves than ECG-gated intermittent triggered imaging. Conversely, the higher-power ECG-gated intermittent imaging appears to provide more intense opacification and fewer motion artifacts than real-time imaging. The determination of the comparative accuracy of the 2 methods in detecting abnormal blood flow is critical before large-scale clinical trials in patients are embarked upon.

This study presents the first data comparing the ability of real-time MCE with triggered imaging to quantify impaired myocardial perfusion and provides the initial quantitative assessment of transmural perfusion gradient by real-time MCE during graded coronary stenoses. The data in this study allow the following conclusions. (1) The ability to visually detect perfusion defects due to graded stenoses does not differ between real-time and triggered imaging. (2) SI versus time plots after bubble destruction by real-time imaging is similar to the SI versus pulsing-interval plots by triggered imaging in the presence of coronary stenoses. (3) With each imaging modality, both the rate of SI rise (b) and plateau intensity (A) decrease progressively as coronary blood flow is incrementally diminished by more severe stenoses, and b is significantly reduced with all grades of stenosis. (4) With each imaging modality, measurements of b and the product of A times b, normalized as the ratio of values in ischemic (LAD) to normal (LCx) coronary perfusion beds, show a good and equivalent correlation with a similar MBF ratio derived from fluorescent microspheres. These data indicate that visual and quantitative analysis of real-time intravenous MCE yield accuracy comparable to that of triggered imaging in the assessment of coronary artery disease.

Comparison of Parameters Derived From Curve Fittings Using 1-Exponential Function Between Real-Time and Triggered Imaging

We compared the quantitative analysis of intensity versus pulsing interval curves by triggered imaging and intensity versus time curves by real-time imaging using a 1-exponential function. Because we used the new harmonic power-pulse inversion technique for both modalities, correlation of MCE-derived parameters with microsphere-derived MBF may not be identical to previous studies performed by...
gray-scale triggered imaging.\textsuperscript{3,8} With both imaging modalities in the present study, however, the product of peak SI and rate of SI rise and the rate of SI rise alone yielded a closer correlation with fluorescent microsphere-derived MBF measures than the plateau SI and will probably be the most valuable criteria for clinical abnormality. Furthermore, the present study demonstrates that although the LAD/LCx ratio of b with real-time imaging (no stenosis 1.02, mild NFLS 0.70, moderate NFLS 0.49, severe NFLS 0.37, FLS 0.18) was different from that with triggered imaging (no stenosis 1.06, mild NFLS 0.58, moderate NFLS 0.46, severe NFLS 0.31, FLS 0.27), the difference was statistically insignificant (Figure 5). Therefore, the magnitude of changes in b values observed with real-time imaging was similar to that with triggered imaging. This finding is not surprising and confirms that both triggered imaging with incremental pulsing intervals and real-time imaging measure microbubble replenishment rate after bubble destruction and have similar accuracy for quantification of MBF. In the present study, although twice the dose of contrast agent and a low dynamic range were applied to achieve myocardial opacification during real-time imaging, myocardial SI was less than during triggered imaging. Thus, clinical application of real-time imaging may be expected to require twice the amount of contrast agent as triggered imaging to achieve comparable diagnostic results.

Transmural Distribution of Myocardial Perfusion

The ability of intermittent triggered B-mode gray-scale MCE to determine the ratio of endocardial/epicardial MBF has been established.\textsuperscript{8} No data exist, however, regarding the ability of real-time MCE to assess the ratio of endocardial/epicardial MBF. In the present study, identification of disturbed transmural distribution of myocardial perfusion by visual examination was better with real-time imaging than with triggered imaging (Figures 2 and 3). Abnormal endocardial/epicardial ratios were particularly apparent with severe stenosis (severe NFLS and FLS) by real-time imaging (Figure 7). In addition, the ratio of endocardial/epicardial parameters derived from real-time MCE at baseline and at each reduced flow state correlated more closely with the ratio of endocardial/epicardial MBF measured by microspheres than the same ratio derived from triggered MCE (Figure 8). This difference may be explained by spatial resolution, which appeared to be lower with triggered imaging by color-coded power-pulse inversion (Figure 2) than real-time imaging (Figure 3). Linka et al\textsuperscript{8} reported that the correlation between the endocardial/epicardial ratios of parameters by gray-scale triggered MCE and microsphere-derived MBF was better for \( A \times b (r=0.88) \) than \( A (r=0.46) \) or \( b (r=0.69) \) alone. We did not use gray-scale imaging, which might have improved the cor-
relation of the endocardial/epicardial ratio by triggered imaging in the present study. Our data, however, clearly establish that real-time MCE can also estimate the endocardial/epicardial ratio of MBF. This ability to depict transmural distribution of myocardial perfusion may be of value in the detection and quantitative assessment of coronary stenoses by clinical MCE.

Methodological Considerations
The present study was performed in open-chest dogs. Although ultrasound attenuation and artifact induced by the closed-chest setting in clinical situations may alter the findings to some degree, we believe that the principles established in this study remain valid. We used a single type and dose of contrast agent (BR1). The dose of agent used was adequate to opacify the imaging field and allow determination of replenishment characteristics. The values obtained also depend on the instrument and settings (such as mechanical index) used, which determine the degree of microbubble destruction. In addition, we analyzed videointensity data rather than digital data for triggered imaging because the feasibility of assessment of myocardial perfusion by triggered imaging has been established by measurements of videointensity from videotape in numerous previous studies.1,3,6,8 Systematic comparison of videointensity and digital data in a subset of 4 animals yielded similar results. The precise results obtained with other machines, settings, and methods for measuring SI might differ. These data, however, have established the basis for the relationship of microbubble replenishment rate into myocardium and lesion severity in real-time and triggered imaging. Finally, in this protocol we examined only stenoses in an anterior location and did not apply other vasodilator stimuli except adenosine. Because attenuation or lateral dropout was sometimes seen with real-time imaging similar to that seen with triggered imaging, assessment of myocardial perfusion of lateral and posterior wall may be problematic.

Clinical Implications
Consensus is still lacking as to the optimal approach to acquiring MCE images to evaluate coronary artery disease. Although low-energy real-time imaging is easy to apply and provides simultaneous wall motion and thickening, it results in less intense opacification and has the potential to introduce tissue signal motion artifacts. Our experimental data demonstrate that microbubble replenishment rate after bubble destruction assessed by MCE provides excellent parameters of regional microcirculatory flow and can be determined equally well by real-time and triggered techniques. These parameters are progressively diminished to an extent similar to that of MBF in the presence of coronary stenoses with both imaging modalities. In addition, disturbed transmural distribution of myocardial perfusion can be identified by both methods. Thus, real-time MCE measurements have the potential to provide microcirculatory data comparable to triggered imaging and yield simultaneous assessment of wall motion abnormalities.

References
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_Circulation_. 2001;104:1550-1556
doi: 10.1161/hc3801.095694
_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2001 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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