Observation of the Ischemic Cascade in Humans Using Contrast Echocardiography During Dobutamine Stress

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Experimental studies have postulated the ischemic cascade and the present study was designed to elucidate whether it can be observed in the clinical setting. Fifty-three patients suspected of having coronary artery disease were studied. Myocardial perfusion abnormalities (MPA) and wall motion abnormalities (WMA) were assessed simultaneously by infusion of Levovist during dobutamine stress echocardiography. Time-intensity data of myocardial opacification were fitted for $Y=A \cdot (1-e^{-kt})$ from which the rate of increase ($k$) of intensity were derived both at rest and during stress. Wall motion was also given a score. Bright opacification was observed in 50 patients: 25 showed significant stenosis (>50%) in the left anterior descending artery (group II) on coronary angiography and 25 did not (group I). Significant differences were found in the $k$ ratio (stress/rest) between the 2 groups at a low-dose (2.0±0.3 vs 1.5±0.5, p<0.05) and at a high-dose of dobutamine (2.7±1.0 vs 1.1±0.5, p<0.001), whereas the wall motion score differed only at a high-dose. Of the 25 patients in group II, MPA preceded WMA in 12, both occurred at the same stage in 12, and neither MPA nor WMA was seen in 1. These data prove the ischemic cascade clinically, using contrast echocardiography, by demonstrating that MPA precede WMA during dobutamine stress in patients with coronary stenosis. (Circ J 2003; 67: 406–410)

Key Words: Contrast echocardiography; Coronary artery disease; Ischemic cascade

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circulation is the cumulative impact of a sequence of pathophysiologic events; reduced perfusion, a decline in function, abnormal electrocardiogram (ECG) changes and, occasionally, angina pectoris occur in quick succession. This sequence of events has been termed the ‘ischemic cascade’ and a recent study has demonstrated it in animal models by showing that perfusion abnormalities precede functional abnormalities under ischemic conditions.

Because the microvascular rheology of the microbubbles used for myocardial contrast echocardiography (MCE) is similar to that of red blood cells, MCE can noninvasively and accurately quantify myocardial perfusion. The diagnostic value of dobutamine stress echocardiography (DSE) for detecting coronary artery disease by estimating wall motion abnormalities (WMA) has been firmly established by 2-dimensional (D) echocardiography provides high-resolution images of cardiac movements as well as detailed functional information.

However, few clinical studies have evaluated myocardial perfusion and contractility simultaneously and so the present study aimed to compare the myocardial perfusion and wall motion abnormalities and to clarify whether myocardial perfusion abnormalities (MPA) precede WMA during dobutamine stress by using MCE and 2-D contrast echocardiography in the clinical setting.

Methods

Study Population

A total of 53 patients (age 46–80 years, mean: 64±7 years; 40 men) who were suspected of having angina pectoris were prospectively studied. All stress studies were performed to evaluate exertional chest pain or shortness of breath. Patients with heart failure, unstable angina, myocardial infarction, congenital or valvular heart disease, cardiomyopathy, abnormal resting left ventricular function, left bundle branch block or arrhythmia were excluded. Of the 53, 26 patients had symptoms suggestive of angina and 22 had undergone prior coronary revascularization. Those taking $\beta$-blockers were asked to gradually stop 24 h before the imaging protocol, whenever possible. All patients gave written informed consent to participate in this study.

Dobutamine Stress Echocardiography

A Sonos 5500 system (Philips Medical Systems) and second harmonic imaging was employed for the study, using an S3 probe, which transmits ultrasound at a mean frequency of 1.6 MHz while receiving at 3.2 MHz. All images for wall motion analysis were obtained during Levovist infusion. The ECG was monitored continuously and blood pressure was measured every minute with an automatic sphygmomanometer. Levovist (Schering AG, Berlin, Germany) was administered intravenously by continuous infusion at 2–3 ml/min (600–900 mg/ml) after a 1 ml bolus injection. After baseline cine loops of 4- and 2-chamber views, and apical long axis views, were recorded, dobu-
Dobutamine was administered in 5-min stages at an infusion rate of 10, 20 and 40 μg/kg per min. Cine loops of the apical views were acquired digitally at each stage. Patients who did not achieve 85% of their age-predicted maximal heart rate were given intravenous atropine (<1 mg), and dobutamine infusion was continued for up to 3 more min. The end-points for termination of the test were the attainment of target heart rate, completion of the study protocol, development of severe ischemia (increasing angina, extensive wall motion abnormality, >3-mm ST segment shift) or the occurrence of intolerable side effects. Other reasons for terminating the test included severe palpitation, dyspnea, significant arrhythmia, severe hypertension or hypotension (>30-mm Hg decrease in systolic pressure). WMA were assessed in the left anterior descending coronary artery territory subtended by each stenosis with coronary angiography was performed in all patients using a standard technique. The coronary artery segment of interest was selected, and the axis and edges of the artery were automatically defined. Measurements were expressed as a percentage of diameter narrowing compared with the diameter of the native vessel. The automated edge detection methodology was visually inspected to ensure that lumen edges were correctly identified. Coronary narrowing of more than 50% diameter in the native vessel was used as the cut-off value. The interventional cardiologist who did not know the clinical data estimated the coronary artery territory subtended by each stenosis with MCE imaging or wall motion. In all 53 patients, the LAD distribution supplied the antero-septal and apical regions.

**Table 1 Hemodynamics During Dobutamine Stress**

<table>
<thead>
<tr>
<th>Group I (&lt;50%; n=25)</th>
<th>Group II (&gt;50%; n=25)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stenosis, %</td>
<td>23±16</td>
<td>83±15</td>
</tr>
<tr>
<td>HR (rest), beats/min</td>
<td>62±13</td>
<td>63±9</td>
</tr>
<tr>
<td>HR (low), beats/min</td>
<td>77±20</td>
<td>79±18</td>
</tr>
<tr>
<td>HR (high), beats/min</td>
<td>121±20</td>
<td>114±22</td>
</tr>
<tr>
<td>SBP (rest), mmHg*</td>
<td>135±14</td>
<td>139±20</td>
</tr>
<tr>
<td>SBP (low), mmHg*</td>
<td>166±29</td>
<td>171±29</td>
</tr>
<tr>
<td>SBP (high), mmHg*</td>
<td>184±28</td>
<td>177±33</td>
</tr>
</tbody>
</table>

Values are mean±SD. HR rate; SBP, systolic blood pressure. *Significant differences between stages.

**Table 2 Data From Myocardial Contrast Echocardiography and Wall Motion Analysis**

<table>
<thead>
<tr>
<th>Group I (&lt;50%; n=25)</th>
<th>Group II (&gt;50%; n=25)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>WMA score (low)</td>
<td>1.1±0.44</td>
<td>1.3±0.43</td>
</tr>
<tr>
<td>WMA score (high)</td>
<td>1.0±0.20</td>
<td>2.0±0.78</td>
</tr>
</tbody>
</table>

WMA, mean microbubble velocity; WMA ratio, WMA at dobutamine stress/WMA at rest; low, low dose of dobutamine; high, high dose of dobutamine. *Significant differences between stages.

**Dobutamine Stress Myocardial Contrast Echocardiography**

Intermittent harmonic imaging was performed with an S3 transducer that transmits ultrasound at a mean frequency of 1.3 MHz and receives it at a mean frequency of 3.6 MHz (Ultra harmonics). The transmit power was set at maximum, and compression was set at 50 dB. The gain setting was adjusted at the beginning of the rest study and held constant thereafter. The apical 4-chamber view, in which the region of interest was placed at the apical-septum, was obtained during end-systolic ECG triggering. The interval between the ECG triggers (pulsing interval: PI) was increased from every heart beat to 2, 3, 4, 8, and 12 cardiac cycles to allow incremental microbubble replenishment. MCE images were obtained at baseline and low-dose and peak-dose dobutamine. The low-dose dobutamine stage was defined as 20 μg/kg per min except if WMA occurred at 10 μg/kg per min, in which case we defined 1 μg/kg per min as low dose. All MCE images were saved digitally at each dobutamine stage and transferred to an off-line computer for analysis.

MCE was performed during continuous infusion of Levovist. Recordings were obtained 2 min after initiating infusion to ensure that myocardial opacification had reached plateau intensity. PI versus background-subtracted myocardial signal intensity were generated and fitted to an exponential function: \( Y = A (1 - e^{-kt}) \) at each dobutamine stage, where \( Y \) is the signal intensity at a PI, \( A \) is the peak plateau amplitude that reflects microvascular cross-sectional area or myocardial blood volume, and \( k \) is the rate of signal intensity rise (slope of the curve) that reflects myocardial microbubble velocity. The product of \( A \) and \( k \) provides a measurement of myocardial blood flow.

**Coronary Angiography**

Coronary angiography was performed in all patients using a standard technique. The coronary artery segment of interest was selected, and the axis and edges of the artery were automatically defined. Measurements were expressed as a percentage of diameter narrowing compared with the diameter of the nearest apparently normal region. The automated edge detection methodology was visually inspected to ensure that lumen edges were correctly identified. Coronary narrowing of more than 50% diameter in the native vessel was used as the cut-off value. The interventional cardiologist who did not know the clinical data estimated the coronary artery territory subtended by each stenosis with MCE imaging or wall motion.

**Protocol**

At baseline, Levovist was administered continuously. Cine loops of 2 cardiac cycles from the apical views were acquired digitally for wall motion analysis, and then MCE images were acquired continuously for myocardial perfusion analysis. After wall motion and myocardial perfusion images were acquired at baseline, dobutamine was infused in 5-min stages of 10, 20, 40 μg/kg per min. At low-dose and peak-dose of dobutamine, wall motion and perfusion analyses were repeated in the same manner as at baseline.

**Statistical Analysis**

Data was presented as mean±standard deviation. Comparisons between stages were made using repeated-measure ANOVA. When differences were found, interstage comparisons were confirmed using Student’s t test. Sensitivity and specificity were calculated by the standard formulas. The comparisons of sensitivity and specificity were made using McNemar’s chi-square test. Categoric data were compared by means of the \( \chi^2 \) or Fisher exact test. Differences were considered significant at \( p<0.05 \) (2-sided). Interobserver and intraobserver variability were determined by the analysis of 20 random patients by 2 independent blinded observers, and by the same observer at 2 different time points.
Table 3  Sensitivity and Specificity of Myocardial Perfusion Abnormalities (MPA) and Wall Motion Abnormalities (WMA)

<table>
<thead>
<tr>
<th></th>
<th>Low</th>
<th>WMA</th>
<th>High</th>
<th>MPA</th>
<th>WMA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity (%)</td>
<td>76 (19/25)</td>
<td>40 (10/25)</td>
<td>84 (21/25)</td>
<td>72 (18/25)</td>
<td></td>
</tr>
<tr>
<td>Specificity (%)</td>
<td>84 (21/25)</td>
<td>92 (23/25)</td>
<td>96 (24/25)</td>
<td>90 (24/25)</td>
<td></td>
</tr>
<tr>
<td>Accuracy (%)</td>
<td>80 (40/50)</td>
<td>66 (32/50)</td>
<td>90 (45/50)</td>
<td>84 (42/50)</td>
<td></td>
</tr>
</tbody>
</table>

Low, low dose of dobutamine; high, high dose of dobutamine.

Results

Patients

MCE images could not be analyzed in 3 patients because of image artifacts, so the results from the remaining 50 patients are presented. Quantitative angiography showed ≤50% stenosis in the LAD coronary artery in 25 patients (group I) and >50% stenosis in 25 patients (group II). In group II, 15 patients had single-vessel disease (SVD) and 10 had multivessel disease (MVD). No differences were noted in the numbers of patients with diabetes mellitus, hypertension and hyperlipidemia between groups I and II.

MCE Data, Wall Motion Analysis and Hemodynamics

Hemodynamics, and MCE data acquired at rest, at low- and high-dose dobutamine from all patients are shown in Tables 1 and 2. No differences in heart rate or blood pressure were noted between the 2 groups at any dobutamine dose. There was a significant interaction effect between the 2 groups over low-dose and high-dose dobutamine. The value of A was similar between the 2 groups at rest, and at low- and high-dose dobutamine (11±1.6 vs 12±2.0; p=0.24, 11±1.4 vs 12±1.8; p=0.82, 12±1.3 vs 11±2.1; p=0.49, respectively). Although there was no difference in the value of \( \bar{a} \) between the 2 groups at a low dose of dobutamine, at a high dose the value was significantly lower in group II than in group I. When the MCE derived \( \bar{a} \) ratio was defined as the value obtained at dobutamine stress divided by that at rest, the MCE derived \( \bar{a} \) ratio was significantly lower in group II than in group I not only at a high dose but also at a low dose of dobutamine. Wall motion score at rest was 1.0 in both group I and group II; the wall motion score was significantly higher in group II than in group I only at a high dose.

For predicting coronary narrowing of more than 50% diameter using the MCE derived \( \bar{a} \) ratio, the best cut-off value derived from the receiver-operating characteristic curve was 1.8 at the low dose and 1.6 at the high dose of dobutamine. When MPA was defined as an MCE derived \( \bar{a} \) ratio of lower than 1.8 at a low dose and 1.6 at a high dose of dobutamine, the sensitivity of MPA was significantly higher than that of WMA at a low dose of dobutamine (p=0.0077) for the presence of coronary narrowing >50% diameter in the LAD territory (Table 3).

MCE images of the apical 4-chamber view from a patient in each of group I and group II obtained at rest and a low and high dose of dobutamine are shown in Fig 1A,B. The corresponding PI versus SI curves from the LAD beds are also shown in Fig 1C,D. In the patient from group I, neither perfusion abnormality nor wall motion abnormality were detected at dobutamine stress, whereas in the patient of group II, although wall motion was normal, a perfusion abnormality was detected (arrows) at a low dose of dobutamine.

Relation Between Perfusion Abnormality and Wall Motion Abnormality

Of the 25 patients in group II, MPA were seen before WMA in 12 (48%), concurrent in 12 (48%) and the remain-
ing 1 patient (4%) developed neither MPA nor WMA. No patient showed WMA before the development of MPA. All patients who had MPA without WMA at a low dose of dobutamine showed WMA at a high dose.

In 15 patients who had SVD, 10 had a preceding occurrence of MPA and 5 did not, and in the 10 patients who had MVD, 2 had a preceding occurrence of MPA and 8 did not. In 19 patients who had ≥75% diameter stenosis in the LAD, 8 had a preceding occurrence of MPA and 11 did not, and in the 6 patients who had between ≥50% and <75% diameter stenosis, 4 had preceding occurrence of MPA and 2 did not. The number of cases of SVD was significantly higher than MVD (p=0.041), whereas there were no differences in the severity of stenosis between the number of patients with preceding MPA and those without (p=0.38). Thus, in this study, we could not find a significant relationship between a preceding occurrence of MPA and the severity of stenosis in the LAD.

Observer Variability
The interobserver and intra-observer variability for the value of A was r=0.80, p<0.01 and r=0.85, p<0.01, respectively.

Discussion
This is the first report to show the ischemic cascade in the clinical setting by demonstrating that MPA preceding WMA during dobutamine infusion.

Assessment of MPA by MCE
MCE is an imaging method that uses intravenously injected microbubbles as red blood cell tracers during simultaneously performed ultrasound imaging. Because the microvascular rheology of the microbubbles used for MCE is similar to that of red blood cells, MCE can give an accurate assessment of myocardial perfusion clinically as well as experimentally. Conventional B-mode ultrasound imaging modified to ultra harmonic mode has been a major innovation that has established the potential of MCE for the assessment of myocardial perfusion. Measurement of the time course of contrast intensity provides a method of quantifying coronary blood flow and thereby identifying and quantifying coronary stenosis. Wei et al fitted the time intensity data to an exponential curve, \( Y = A \times e^{-\frac{t}{\text{time}}}, \) where A is the plateau signal intensity representing myocardial blood volume and \( \text{time} \) is the rate of rise of signal intensity reflecting the mean microbubble velocity. In open-chest animal models, measurement of A and \( \text{time} \) before and after the infusion of dobutamine provides an assessment of the severity of coronary stenosis. Intravenous MCE enables a quantitative, non-invasive and physiological assessment of myocardial perfusion in the clinical setting. The results of the present study showed the value of \( \text{time} \) had advantages over the value of A for assessment of myocardial ischemia, for the following reasons. First, microbubble concentration has to be the same between rest and during dobutamine stress to compare the A value, which can not be guaranteed in the clinical situation even with a continuous infusion. The plateau myocardial signal intensity is affected by small changes in the blood concentration of microbubbles, whereas the microbubble velocity (the value of \( \text{time} \)) is not affected. Second, artifacts are more likely to cause a decrease in backscatter from microbubbles than in an experimental model. Thus, as shown from the current study, measuring the rate of rise rather than the plateau of myocardial signal intensity is a more robust and accurate estimate of coronary stenosis with MCE in the clinical setting.

The Ischemic Cascade
When a coronary artery is occluded, reduced perfusion, a decline in function, and an abnormal ECG occur in quick succession; a phenomenon that has been termed the ‘ischemic cascade’. It is the sequence of events that occurs when there is an imbalance between myocardial oxygen supply and demand. In experimental models, the ischemic cascade has been shown by regional perfusion abnormalities preceding regional function abnormalities during demand ischemia. In the present study, myocardial perfusion was assessed by MCE and functional analysis was performed with 2-D echocardiography. The use of a contrast agent during DSE improves endocardial border visualization, allowing accurate wall motion analysis. Though wall motion and MCE images were in agreement in 90% of the coronary artery territories at a high dose of dobutamine, there were a significant number of territories subtended by >50% diameter stenosis, which exhibited a low A ratio despite a normal wall motion score at a low dose. In such segments, all of the perfusion abnormalities at a low dose of dobutamine were followed by WMA at higher doses.

In the present study, we have shown clinically that abnormal perfusion precedes abnormal function and that perfusion abnormality has a higher sensitivity for the detection of coronary stenosis compared with WMA at a low dose of dobutamine stress. These findings are expressions of the ischemic cascade of ischemia and infarction, with WMA at a high dose of dobutamine as the cause of functional abnormalities and that a perfusion abnormality does occur as a result of disproportionate perfusion distribution, not of actual ischemia. The existence of MVD could affect the sequence of MPA and WMA (i.e., the ischemic cascade). In the experimental study, the spatiotemporal discordance between MPA and WMA was significantly larger in SVD than in MVD, which indicates that MPA precedes WMA in SVD. On the other hand, the occurrence of WMA was influenced by the severity of the stenosis in SVD. Therefore, theoretically, MPA likely precedes WMA in SVD, especially with relatively mild stenosis. In the present study, the number cases of SVD was significantly higher than MVD in which MPA preceded WMA and there was no significant relationship between the severity of LAD stenosis and the occurrence of MPA. These results are compatible with the finding from the experimental study that discordance between MPA and WMA was significantly greater in SVD than in MVD at the same time and supports that the occurrence of a perfusion abnormality before a wall motion abnormality is irrespective of stenosis severity.

Study Limitations
This method was only applied to the LAD lesion for 2 reasons. First, myocardial opacification at the apical-septum is accurate and therefore suitable for quantified assessment of perfusion in the clinical setting. Secondly, assessment of WMA in the LAD territory was accurate. Whether this method is applicable to the other coronary artery lesions remains to be determined.

Acquisition of intermittent MCE data is currently tedious and time consuming, and MPA could not be determined at the same time as WMA in the strict sense of the
Clinical Implications

This diagnostic approach, dobutamine stress MCE, has potential in several clinical conditions. When a perfusion abnormality is detected at a lower dose, it is not necessary to increase the dose of dobutamine and thus hazardous complications can be avoided. It should also be helpful for wall motion analysis at a low dose as well as at a high dose of dobutamine. It can be used for detecting coronary stenosis in some conditions such as left bundle branch block, in which assessment of wall motion alone is difficult or unreliable. The ability to quantify MCE rapidly and serially during multiple interventions could make it a remarkably valuable tool for clinicians to evaluate coronary stenosis and coronary flow reserve. If it is performed correctly, it has significant advantages over radionuclide imaging, such as significantly better spatial and temporal resolution, reduced cost and the technical ease with which it can be performed.

Conclusion

Our data have shown that the ischemic cascade can be identified in the clinical setting by demonstrating that perfusion abnormalities precede wall motion abnormalities during DSE.

Acknowledgment

We thank Miss Helen Bunten for constructive comments.

References


