Changes in transmural distribution of myocardial perfusion assessed by quantitative intravenous myocardial contrast echocardiography in humans

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Objective: To clarify whether changes in transmural distribution of myocardial perfusion under significant coronary artery stenosis can be assessed by quantitative intravenous myocardial contrast echocardiography (MCE) in humans.

Methods: 31 patients underwent dipyridamole stress MCE and quantitative coronary angiography. Intravenous MCE was performed by continuous infusion of Levovist. Images were obtained from the apical four chamber view with alternating pulsing intervals both at rest and after dipyridamole infusion. Images were analysed offline by placing regions of interest over both endocardial and epicardial sides of the mid-septum. The background subtracted intensity versus pulsing interval plots were fitted to an exponential function, $y = A(1 - e^{-\beta t})$, where A is plateau level and β is rate of rise.

Results: Of the 31 patients, 16 had significant stenosis (> 70%) in the left anterior descending artery (group A) and 15 did not (group B). At rest, there were no differences in the A endocardial to epicardial ratio (A-EER) and β -EER between the two groups (mean (SD) 1.2 (0.6) v 1.2 (0.8) and 1.2 (0.7) v 1.1 (0.6), respectively, NS). During hyperaemia, β -EER in group A was significantly lower than that in group B (1.0 (0.5) v 1.4 (0.5), $p < 0.05$) and A-EER did not differ between the two groups (1.0 (0.5) v 1.2 (0.4), NS).

Conclusions: Changes in transmural distribution of myocardial perfusion under significant coronary artery stenosis can be assessed by quantitative intravenous MCE in humans.

In the presence of major coronary artery stenosis, reduction in coronary blood flow causes a shift in transmural myocardial blood flow (MBF) distribution towards the epicardium, producing myocardial ischaemia predominantly in the endocardium.^{1,2} Thus, the ability to image transmural distribution of MBF may provide a non-invasive estimation of the severity of coronary artery stenosis.

Quantitative intravenous myocardial contrast echocardiography (MCE) is a new way to assess myocardial perfusion non-invasively.^{3–8} MCE allows us to assess regional MBF because of a high axial resolution in comparison with conventional methods for assessment of myocardial perfusion (single photon and positron emission tomography). In previous experimental studies, changes in transmural distribution during myocardial ischaemia were well visualised by intravenous MCE.^{9–11} However, MCE has not been well documented in the clinical setting.

Recent studies have shown that quantitative assessment of myocardial opacification obtained by intravenous MCE yields a parameter representing MBF velocity, which diminishes incrementally in the presence of more severe coronary artery stenosis.^{12–15} Therefore, the purpose of this study was to clarify whether changes in transmural distribution of myocardial perfusion caused by pronounced coronary artery stenosis can be assessed by quantitative intravenous MCE in humans.

METHODS

Patient population

We selected 31 patients (25 men, six women, mean (SD) age 62 (10) years) with suspected or known coronary artery disease for this study. These patients were scheduled for dipyridamole stress intravenous MCE and diagnostic cardiac catheterisation. Exclusion criteria were prior myocardial infarction, prior coronary artery bypass graft, non-sinus rhythm,

significant stenosis (> 70% diameter stenosis) in the circumflex or right coronary artery, and inadequate visualisation on echocardiography. The mean interval between MCE and diagnostic cardiac catheterisation was 3 (6) days. All patients gave written informed consent for participation in the study.

Intravenous MCE

The contrast agent used in this study was Levovist (Shering AG, Berlin, Germany), which contains galactose based transpulmonary microbubbles. Bubble sizes range from 2–8 μ m, with 97% of the bubbles less than 6 μ m as determined by laser analysis. An intravenous infusion of Levovist (at a rate of 400–600 mg/min with a concentration of 300 mg/ml) was given by injecting extension tubing through a 20 gauge intravenous catheter using the Medrad Pulsar (Medrad Inc, Indianola, Pennsylvania, USA) as an infusion device. The rate was adjusted as needed to maximise the myocardial opacification.

We used a Sonos 5500 (Philips Medical Systems, Andover, Massachusetts, USA) modified for ultraharmonic imaging. The mean transmitting frequency was 1.3 MHz and the mean receiving frequency was 3.6 MHz. The mechanical index was set as high as possible to increase microbubble destruction.

Images were acquired by gating to the ECG at end systolic triggering. During the initial two minutes of Levovist infusion, we optimised the device settings, such as gains, scan planes, and infusion rates of the agent, and held them constant for

Abbreviations: EER, endocardial to epicardial ratio; LAD, left anterior descending artery; MBF, myocardial blood flow; MCE, myocardial contrast echocardiography

subsequent image acquisitions. Images were obtained at pulsing intervals from 1–8 cardiac cycles. Dipyridamole was then infused at a rate of 0.56 mg/kg/min over four minutes and images were acquired in the same manner after dipyridamole infusion.

Images with MCE were digitally captured and analysed. A region of interest (about 300 square pixels) was placed over the endocardial and epicardial half of the mid-septum and the background subtracted intensity was measured at each pulsing interval because myocardial perfusion can be assessed without artefacts and attenuation in this territory. We made every effort not to include right ventricular trabeculation in the region of interest. Grey scales software (QuantiCon, Echo-Tech 3D Imaging Systems GmbH, Hallbergmoos, Germany), which permits quantification of signal intensity (0–255 scale), was used to measure the contrast intensity.

Plots of the background subtracted intensity of the region of interest versus each pulsing interval were generated and fitted to an exponential function, $y = A(1 - e^{-\beta t})$, to obtain the plateau level (A) and rate of rise (β) of background subtracted intensity both at rest and after dipyridamole infusion. Each value of A and β of the endocardium was divided by that of the epicardium and the results were defined as A endocardial to epicardial ratio (A-EER) and β endocardial to epicardial ratio (β -EER). The analysis was performed without knowledge of the angiographic findings.

Coronary angiography

Coronary angiography was performed in all patients by using standard techniques. Coronary stenosis was evaluated in multiple projections by one investigator who had no knowledge of MCE results. The percentage diameter stenosis was analysed quantitatively using CMS analysis software (Medical Imaging System).¹⁶ Coronary stenosis was considered if there was > 70% diameter stenosis on quantitative coronary angiography.

Statistical analysis

Values are expressed as mean (SD). The two study groups were compared for the parametric data with an unpaired *t* test. Differences between proportions were assessed by χ^2 analysis. Echocardiographic and haemodynamic variables after dipyridamole infusion of the two groups were evaluated by two way repeated measures analysis of variance, testing for group effect, dipyridamole effect, and interaction. Fisher's protected least significant difference test was used for post hoc testing. Differences were considered significant at $p < 0.05$. Interobserver and intraobserver variabilities for measurement of the value of A and β were obtained by analysis of 10 random stages by two independent blinded observers and by the same observer at two different time points. Correlations were obtained using least squares fit linear regression analysis.

RESULTS

All 31 patients underwent intravenous MCE and diagnostic cardiac catheterisation without clinical events. Table 1 shows clinical characteristics and angiographic data in these 31 patients. No significant difference in age, sex, or any coronary risk factors was found between the two groups.

Coronary angiography

Sixteen patients exhibited significant coronary stenosis in the left anterior descending artery (LAD) (group A) and 15 did not (group B). On coronary angiography, percentage diameter stenosis of the LAD in group A was greater than that in group B (83 (14)% *v* 41 (20)%, $p < 0.0001$) (table 1). Three patients had total occlusion in the proximal LAD, with good collateral flow from the right coronary artery.

Dipyridamole stress intravenous MCE

Of the 31 patients, five (16%) developed chest discomfort during dipyridamole infusion and one (3%) developed ST depres-

Table 1 Clinical characteristics and angiographic data

	Group A (n=16)	Group B (n=15)	p Value
Mean (SD) age (years)	60 (10)	64 (11)	NS
Male sex	12 (75%)	13 (87%)	NS
Hypertension*	5 (31%)	8 (53%)	NS
Hypercholesterolaemia**	10 (62%)	8 (53%)	NS
Diabetes	3 (19%)	5 (33%)	NS
Smoking	6 (38%)	6 (40%)	NS
Mean (SD) stenosis (% diameter)	83 (14)	41 (20)	<0.0001

*Blood pressure >150/95 mm Hg in repeated measures; **Total cholesterol >6mmol/l.

Table 2 Haemodynamic data in groups A and B

	Group A (n=16)	Group B (n=15)
Heart rate (beats/min)		
Baseline	64 (11)	64 (9)
Hyperaemia	74 (12)	73 (11)
Blood pressure (mm Hg)		
Baseline	131 (19)	133 (25)
Hyperaemia	129 (24)	124 (23)

Data are mean (SD).

sion without angina. No adverse effects were noted at rest or after dipyridamole infusion. Table 2 shows haemodynamic data. After dipyridamole infusion, heart rate increased significantly ($p < 0.0001$). However, two way analysis of variance showed no significant interaction in terms of heart rate and blood pressure between the two groups during dipyridamole infusion.

Figure 1 illustrates MCE images both at rest and after dipyridamole infusion from a patient without significant stenosis in the LAD. Normal myocardial perfusion was observed with MCE both at rest and after dipyridamole infusion. Figure 2 is from a patient with significant stenosis in the LAD. At rest, the MCE image was similar to that shown in fig 1, with a similar microbubble velocity. After dipyridamole infusion, myocardial perfusion abnormalities were observed in the endocardium with MCE at short pulsing intervals, resulting in lower microbubble velocity in the former. Perfusion abnormalities with MCE at short intervals were decreased with prolonged pulsing intervals. In this instance, the peak intensity was similar at rest and after dipyridamole infusion.

Table 3 also shows the results of MCE. At baseline, there were no significant differences in parameters measured by MCE between groups A and B (table 3). Two way analysis of variance showed no significant differences or interaction in terms of A epicardium, A endocardium, and β epicardium. However, two way analysis of variance showed an effect on β endocardium after dipyridamole infusion ($p = 0.006$). β Endocardium after dipyridamole infusion in group B increased significantly compared with that in group A. Thus, at rest, there were no differences in A-EER and β -EER between the two groups (1.2 (0.6) *v* 1.2 (0.8) and 1.2 (0.7) *v* 1.1 (0.6), respectively, NS; table 4). After dipyridamole infusion, β -EER in group A was significantly lower than that in group B (1.0 (0.5) *v* 1.4 (0.5), $p < 0.05$), while A-EER did not differ between the two groups (1.0 (0.5) *v* 1.2 (0.4), NS; table 4).

The interobserver and intraobserver variabilities for the values A and β derived from exponential function curves from 10 separate random stages were excellent: $r = 0.87$, $p < 0.01$, and $r = 0.90$, $p < 0.01$, respectively, for A, and $r = 0.89$, $p < 0.01$, and $r = 0.91$, $p < 0.01$, respectively, for β .

Baseline

Hyperaemia

Figure 1 Myocardial contrast echocardiographic (MCE) images at baseline (upper) and during hyperaemia (lower) in group B. The corresponding quantitative MCE data for the endocardium (triangles) and epicardium (squares) are shown on the right.

DISCUSSION

We present the first data showing that intravenous MCE permits assessment of transmural distribution of myocardial perfusion in the clinical setting. In addition, we quantitatively assessed myocardial perfusion with MCE using the exponential curve $y = A(1 - e^{-\beta t})$.

Transmural distribution of myocardial perfusion

Controversy continues regarding the ability of MCE, which uses intracoronary injection of microbubbles, to determine the MBF EER.¹⁷ However, experimental studies indicate that intravenous MCE can be used to assess transmural distribution of myocardial perfusion.⁹⁻¹¹ Linka and colleagues⁹ measured myocardial perfusion with harmonic B mode in the layers of the myocardium and obtained a good correlation between MCE derived and microsphere derived EER. Masugata and colleagues¹⁰ and Villanueva and associates¹¹ reported that identification of disturbed transmural distribution of myocardial perfusion was possible with harmonic power Doppler imaging. As a consequence, quantitative assessment of MBF velocity by intravenous MCE has been evaluated sufficiently to determine its usefulness for assessing transmural distribution of myocardial perfusion in the experimental setting.

In our protocol, we used ultraharmonic imaging and digital analysis. Traditional grey scale imaging depends primarily on the increased backscatter and resonance phenomenon of microbubbles to produce a contrast signal and does not readily differentiate reflections produced by tissue from those of the contrast agent. Ultraharmonic imaging produces low pre-contrast tissue signals and high post-contrast myocardial opacification. Therefore, this tool has a greater sensitivity for detecting microbubbles and can readily distinguish bubble signals from those generated by tissue.¹⁵ Another methodological advantage of ultraharmonic imaging is its high reso-

lution and the ability to avoid motion artefacts compared with harmonic power Doppler imaging. Moreover, images were obtained digitally to avoid loss of data quality inherent in analogue recording. As a consequence, the methods in this study allowed us to assess transmural distribution of myocardial perfusion in the clinical setting.

In the present study, we analysed MCE imaging using the method of Wei and colleagues.¹² In their experimental study, they applied progressively prolonged pulsing intervals and showed that the signal intensity increased over time until a peak plateau was reached, at which point refilling of the imaging field between pulses was complete. They fitted the time intensity data to an exponential curve, $y = A(1 - e^{-\beta t})$, where A is the plateau background subtracted intensity representing myocardial blood volume and β is the rate constant reflecting the rate of rise of BSI (or the mean microbubble velocity). This showed that β was correlated with MBF velocity.

The results of this study showed that MCE indices—that is, A-EER and β -EER—did not differ between patients with and without stenosis at rest. The EER was 1.0–1.2. This is compatible with experimental findings that the flow ratio assessed by microsphere was 1.0–1.5.^{9, 18} During hyperaemia, β -EER was lower in patients with stenosis than in those without stenosis. This result suggests that endocardial blood flow decreases in the presence of coronary stenosis. Recent experimental studies showed that EER diminished incrementally in the presence of more severe coronary artery stenosis.^{9, 10} It is speculated that the endocardium is more susceptible to myocardial ischaemia than the epicardium because increased wall tension causes a relative increase in myocardial oxygen demand.¹⁹⁻²¹

In humans, Wei and colleagues¹³ reported that myocardial ischaemia causes changes in quantitative indices of MCE. Although they insisted that the value of β had advantages over

Baseline

Hyperaemia

Figure 2 MCE images at baseline (upper) and during hyperaemia (lower) in group A. The corresponding quantitative MCE data for the endocardium (triangles) and epicardium (squares) are shown on the right.

Table 3 Myocardial contrast echocardiographic data in groups A and B

	Group A (n=16)	Group B (n=15)
A Epicardium		
Baseline	23.1 (13.2)	23.8 (9.7)
Hyperaemia	22.9 (10.6)	25.5 (8.5)
A Endocardium		
Baseline	25.6 (15.6)	24.5 (12.5)
Hyperaemia	25.2 (13.6)	28.3 (10.2)
β Epicardium		
Baseline	0.81 (0.32)	0.65 (0.29)
Hyperaemia	0.80 (0.49)	0.87 (0.38)
β Endocardium		
Baseline	0.77 (0.21)	0.76 (0.46)
Hyperaemia	0.66 (0.33)	1.19 (0.54)*

Data are mean (SD). *p<0.005 versus group B.

Table 4 Endocardial to epicardial ratio (EER) at baseline and during hyperaemia measured by myocardial contrast echocardiography

	Group A (n=16)	Group B (n=15)	p Value
Baseline			
A-EER	1.2 (0.6)	1.2 (0.8)	NS
β-EER	1.2 (0.7)	1.1 (0.6)	NS
During hyperaemia			
A-EER	1.0 (0.5)	1.2 (0.4)	NS
β-EER	1.0 (0.5)	1.4 (0.5)	<0.05

Data are mean (SD).

the value of A for assessment of coronary blood flow, they did not report quantification of transmural distribution of myocardial perfusion. Accordingly, we sought to determine whether intravenous MCE can be used to assess transmural maldistribution and whether quantitative indices of MCE change with significant coronary artery stenosis. In the present study, we found that β-EER reflects changes in transmural distribution of myocardial perfusion under significant coronary artery stenosis in the clinical setting.

Clinical implications

To our knowledge, this is the first study to show transmural distribution of myocardial perfusion assessed by intravenous MCE in humans. This has at least two potential clinical consequences. Firstly, echocardiography has been widely and readily available not only to cardiologists but also to practitioners.

It is an efficient modality because it is relatively inexpensive, less time consuming, and easily repeatable. In addition, MCE provides simultaneous information of wall motion and myocardial perfusion safely.

Secondly, MCE has better axial resolution than conventional myocardial perfusion imaging such as single photon and positron emission tomography. A previous study reported that MCE in which intracoronary injection of microbubbles is used enables assessment of endocardial ischaemia in humans.¹ The ability to depict transmural distribution of myocardial perfusion in MCE may be of clinical value in the detection and quantitative assessment of coronary stenosis. However, intracoronary injection of microbubbles is invasive. Accordingly, intravenous MCE has permitted non-invasive and physiological assessment of myocardial perfusion in the clinical setting. In addition, we assessed quantitatively myocardial perfusion with MCE. Curve fitting has the potential to reduce noise and provide insight into regional myocardial blood volume (A) and MBF velocity (β) seen under physiological conditions.¹²

Study limitations

There are several limitations to this study. Firstly, we applied the MCE method only to the mid-septum, where myocardial opacification was obtained more successfully, but not to the other segments. Further, investigations are necessary to evaluate whether this method can be applied to other segments.

Secondly, the study involved a relatively small number of selected patients. A large number of patients should be examined in a randomised fashion in future investigations.

Thirdly, there is no ideal for regional myocardial perfusion in the clinical studies because radiolabelled microspheres are not suitable. Commonly used techniques for myocardial perfusion imaging such as single photon and positron emission tomography do not have the spatial resolution to discriminate between endocardial and epicardial MBF.

Lastly, MCE is dependent on microvascular blood flow, which reflects the combined effects of both the epicardial coronary stenosis and microvascular dysfunction on flow reserve. Wei and colleagues¹⁵ showed that MCE parameters decreased in patients with multiple risk factors. Because the prevalence of these conditions was similar in our patients with a different degree of coronary stenosis, our data indicate that intravenous MCE can still be used to assess transmural distribution of MBF.

Conclusions

Changes in transmural distribution of myocardial perfusion under significant coronary artery stenosis can be assessed by intravenous MCE in humans.

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