Experimental Studies

Novel Method for Assessing Myocardial Perfusion

Visualization and Measurement of Intramyocardial Coronary Blood Flow in the Entire Left Ventricular Wall Using Contrast Enhanced, High Frequency Doppler Echocardiography

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SUMMARY

Using a high frequency ultrasonic transducer, intramyocardial coronary blood flow (IM-CBF) can be visualized and evaluated during hemodynamic changes in the anterior wall and septum of the left ventricle (LV). We tested the hypothesis that detection and quantitative measurement of IM-CBF of entire LV segments are feasible using a high frequency ultrasonic transducer in conjunction with intravenous contrast injection *in vivo*.

A 3 - 8 MHz transducer was used to image and measure IM-CBF in 10 anesthetized dogs. We obtained a color Doppler image of IM-CBF in the LV short-axis view after intravenous Levovist(TM) injection (25 mg / ml). The IM-CBF velocity was recorded using spectral Doppler in the antero-septal and infero-posterior wall of closed chest dogs and in the entire LV after opening the chest. A significant increase in IM-CBF velocity was observed in all LV regions after adenosine 5'-triphosphate (ATP) administration. After Levovist(TM) injection, the visualization of IM-CBF was improved and the spectral Doppler pattern of coronary flow velocity was clarified compared to baseline. IM-CBF was assessed in the antero-septal region of the LV before and after left anterior descending coronary artery occlusion.

A high frequency ultrasonic transducer in conjunction with intravenous contrast injection improved IM-CBF visualization, enabling quantitative evaluation of the intramyocardial coronary circulation in the entire LV after coronary occlusion and hyperemia. This study may represent a step towards noninvasive assessment of myocardial perfusion before and after coronary reperfusion. (Jpn Heart J 2001; 42: 101-113)

Key words: Intramyocardial coronary blood flow, Spectral Doppler, Intravenous contrast injection

THE evaluation of color images and flow velocities of major epicardial coronary arteries using transesophageal and transthoracic echocardiography has been

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reported in recent years.¹⁻⁵⁾ Using a newly developed high frequency transducer, the distal left anterior descending artery (LAD) and its coronary blood flow can be detected clinically in echocardiography laboratories. Non-invasive assessment of coronary flow velocity and coronary flow velocity reserve is now possible in patients with ischemic heart disease.^{6,7)} Transesophageal and transthoracic color and pulsed Doppler echocardiography have enabled the visualization of intramyocardial coronary blood flow and measurement of flow velocity in humans.⁸⁻¹⁰⁾ However, intramyocardial coronary blood flow detection is more difficult for further than closer fields owing to attenuation and the relatively small backscatter signal from coronary blood flow. Transpulmonary ultrasonic contrast agents have been developed as good ultrasound reflectors and spectral Doppler signal enhancers in the clinical setting.¹¹⁻¹³⁾ We investigated the feasibility of intramyocardial coronary blood flow evaluation using a high frequency transducer for Doppler echocardiography with contrast enhancement, and defined flow velocities in all segments of the left ventricle (LV).

METHODS

Animal preparation: Ten beagle dogs weighing 10-12 kg were anesthetized with sodium pentobarbital (30 mg / kg i.v.) following a 100 mg bolus injection. After intubation, they were ventilated using room air by a respirator (Respirator model SN-480-3, Murai Seisakusho, Japan), supplemented with 100% oxygen at a rate sufficient to maintain arterial oxygen tension at physiological levels. The right femoral artery and vein were isolated and catheterized and the catheters advanced to the aortic arch and inferior vena cava, respectively. After completing the protocol for the closed-chest study, a midline thoracotomy was performed. The heart was exposed and suspended in a pericardial cradle. The left anterior descending coronary artery (LAD) was isolated and a snare occluder placed proximally. All dogs underwent continuous heart rate and ECG monitoring. Arterial pressure was measured and zeroed at the mid-chest level. This protocol complied with the standards set by the Institutional Board for Animal Study of Osaka City University Medical School.

Echocardiography: Echocardiographic examinations were performed using a commercially available echocardiographic system (Agilent Technologies Sonos 5500, Andover, MA, USA) with a 3 to 8 MHz broad band transducer. Intramural coronary artery blood flow images were obtained from the short-axis view of the LV at the level of the papillary muscles. The transducer was carefully tilted and rotated, using a color Doppler image as a guide, until the diastolic coronary blood flow in the left ventricular

wall was visualized. Color Doppler imaging was performed in a conventional manner. The color flow velocity range (Nyquist limit) was adjusted to a relatively low level (10-20 cm/sec), the Doppler velocity filter set at a high level, and the color persistence and gain settings adjusted to provide optimal intramyocardial coronary flow images. Following identification, coronary blood flow velocity was measured by a pulsed wave Doppler technique. To discriminate small coronary arteries from veins, Doppler color mapping of the flow direction was used. For example, if the coronary flow was directed from the epicardium to endocardium throughout diastole, the imaged vessels were defined as small arteries. The sample volume of a pulsed wave Doppler (width, 3-5 mm) was positioned at the center of the color flow images of the intramyocardial coronary artery. A careful adjustment was made to minimize the angle between the insonified Doppler beam and the flow direction of the intramyocardial coronary artery. Color flow Doppler and pulsed Doppler signals were recorded on super VHS videotapes at a sweep speed of 100 mm / sec. Images were stored in cineloop fashion on magnet optical disks, permitting frame-by-frame review for subsequent analysis. Using measurement software installed in the machine, the spectral Doppler flow velocity pattern was traced manually. Average peak diastolic flow velocity was calculated from at least three cardiac cycles.

Echo-contrast enhancement: An intravenous contrast agent (Levovist, Schering AG, Germany) was used as the Doppler signal enhancer; 1-3 ml of a 25 mg / ml concentration was injected in this study. The contrast medium was prepared manually and injected as a bolus of approximately 1 ml through the right femoral vein followed by a flush of 5 ml of physiologic saline. Contrast agent was added as needed to maintain contrast enhancement.

Measurement of intramyocardial coronary blood flow: All dogs were examined in the right decubitus position when the chest was closed, and in the supine position when the chest was open. Using color Doppler signals as a guide, intramyocardial coronary blood flow was measured in all segments of the LV wall (anterior, septum, inferior, posterior, and lateral) at baseline and after Levovist injection and baseline spectral Doppler signals were obtained in each LV segment and recorded on MO disk and s-VHS tape. Adenosine 5'- triphosphate (ATP, bolus injection of 20 μ g / kg) was administered and spectral Doppler signals from each segment of the entire LV wall were recorded during the vasodilatory response in both closed and open chest conditions. In open chest dogs, the intramyocardial coronary

flow velocity was also recorded after 30 to 120 seconds of LAD occlusion as a reactive hyperemic response of the coronary arteries.

Two experienced investigators analyzed each study. Measurements were performed off-line by tracing the contour of the spectral Doppler signal using the ultrasound system analysis package. Peak average diastolic velocities and time velocity integrals of intramyocardial flow were measured at baseline, 1 minute after ATP administration and at peak hyperemic condition after reperfusion. Data from three cardiac cycles were averaged.

Data analysis: The data are expressed as mean $\pm$ standard deviation. The differences between baseline and hyperemic data were tested using a paired two-tailed Student's *t* test. A probability value of less than 0.05 was considered statistically significant.

RESULTS

Detection and measurement of intramyocardial coronary blood flow: Small intramyocardial coronary arteries and veins are located side by side. Therefore, pairs of color flow Doppler signals traversed the myocardium and were recorded in diastole. Thus arterial flow was defined as the flow from the epicardium to the endocardium during diastole. Representative color Doppler mapping images of intramyocardial coronary blood flow in each LV segment are shown in Figure 1. The intravenous injection of Levovist contrast agent markedly enhanced color Doppler signals and improved the recording of intramyocardial coronary blood flow. A representative case of color and spectral Doppler signal enhancement before and after the Levovist injection is shown in Figure 2. The maximal enhancement by Levovist was observed within 60 seconds after contrast injection.

In the closed chest dogs, intramyocardial coronary blood flow was visualized in the anterior in 7 cases at baseline, 10 cases after Levovist injection, and 9 cases in which a stable phasic intramyocardial coronary blood flow velocity pattern could be recorded by pulsed Doppler. The orders were 8, 10 and 9 in the septum, 3, 7 and 4 in the lateral wall, 2, 6 and 4 in the inferior wall and 2, 6 and 4 in the posterior wall (Table I).

Intramyocardial coronary flow Doppler signals were obtained in 15 of 20 segments near the transducer (anterior wall and septum), and in 7 of 30 segments far from the transducer (inferior, posterior and lateral wall). Using Levovist, detection of the color Doppler signal improved in all 20 near segments and 19 of 30 far segments, totaling 39 of 50 segments (78%). In each segment visualized by color Doppler, it was possible to measure intramyocardial coronary blood flow velocities in 18 of 20 near

Figure 1. Color Doppler flow signals at each segment of the left ventricle in diastole.

Figure 2. Color flow Doppler mapping in the anterior wall of the left ventricle in diastole, showing transmural coronary blood flow. **A:** Color Doppler flow signals traversed the myocardium from the epicardium to endocardium before injection of contrast agent. **B:** After contrast injection.

Table I. Results of the Assessment of Intramyocardial Coronary Artery Flow in Each Segment of the Left Ventricle in the Closed and Open Chest Dogs

	Closed chest			Open chest		
	Baseline	Levovist	Velocity	Baseline	Levovist	Velocity
Anterior	7	10	9	10	10	10
Septum	8	10	9	10	10	10
Lateral	3	7	4	6	10	8
Inferior	2	6	4	5	10	7
Posterior	2	6	4	7	10	10

Baseline, Levovist: number of dogs in which the intramyocardial coronary flow was detected by color Doppler echocardiography velocity: number of dogs in which the intramyocardial coronary flow velocity was measured by pulsed Doppler echocardiography

Table II. Flow Velocities of Intramyocardial Coronary Artery at each Segment of the Left Ventricle in the Closed Chest Dogs.

	Control		ATP	
	Peak (cm/s)	TVI (cm)	Peak (cm/s)	TVI (cm)
Anterior	35.2±7.9	6.9±3.5	62.6±15.4*	7.9±2.5†
Septum	36.7±9.6	7.5±2.2	65.3±14.7*	8.1±2.7
Lateral	41.6±8.1	5.0±2.2	82.2±16.6	12.4±1.3†
Inferior	48.3±5.0	7.0±2.0	78.5±15.5*	9.2±1.1
Posterior	35.9±5.7	5.2±1.8	72.8±32.8*	9.3±4.9

* $p < 0.05$ vs peak velocity at baseline. † $p < 0.05$ vs TVI at baseline.

segments, 12 of 19 far segments, and in 30 of 39 segments visualized by color Doppler (77%). Table II shows the coronary flow velocity and time velocity integral before and after the contrast enhancement of the Doppler signals in closed chest dogs.

The intramyocardial coronary blood flows were successfully visualized by color Doppler in all 10 dogs in the open chest phase of the study. The number of dogs in which stable, phasic intramyocardial coronary blood flow velocity could be recorded by pulsed Doppler was 10 of 10 cases (100%, anterior), 10 / 10 (100%, septum), 8 / 10 (80%, lateral), 7 / 10 (70%, inferior) and 10 / 10 (100%, posterior). Thus, it was possible to measure intramyocardial coronary blood velocities in 45 of 50 segments (90%).

Following LAD occlusion, color Doppler signals from intramyocardial coronary blood flow in the anterior and septal regions faded by

Table III. Flow Velocities of Intramyocardial Coronary Artery at each Segment of the Left Ventricle in the Open Chest Dogs.

	Control		ATP		Hyperemia	
	Peak (cm/s)	TVI (cm)	Peak (cm/s)	TVI (cm)	Peak (cm/s)	TVI (cm)
Anterior	45.6±8.7	8.5±2.3	86.2±19.7*	13.2±2.0 [†]	93.0±33.5*	19.5±6.7 [†]
Septum	44.9±10.8	9.6±3.6	80.3±19.8*	12.6±3.2 [†]	92.7±49.6*	14.2±7.7 [†]
Lateral	45.9±11.0	9.8±3.8	96.1±39.0*	5.3±8.1		
Inferior	43.7±10.5	10.1±4.8	80.1±19.1*	14.6±6.5 [†]		
Posterior	46.0±18.5	10.6±5.1	91.0±42.5*	14.9±8.4 [†]		

* $p < 0.05$ vs peak velocity at baseline. [†] $p < 0.05$ vs TVI at baseline.

degrees. After a 30 second occlusion and the LAD release, the intramyocardial coronary blood flow gradually appeared, and the velocity obtained by pulsed Doppler indicated reactive hyperemia. The intramyocardial coronary blood flow in the risk area was gradually visualized and remarkably increased in the color Doppler image proving a reactive hyperemia in the severe ischemic region. The hyperemic coronary flow velocity was obtained by a pulsed Doppler technique. Table III shows the velocity and time velocity integral before and after contrast enhancement and at reactive hyperemia in open chest dogs.

DISCUSSION

The major findings of this study were: (1) intramyocardial coronary blood flow was visualized in the entire LV by using contrast-enhanced color Doppler echocardiography with a high frequency transducer; (2) intramyocardial coronary flow velocity could be measured by pulsed wave Doppler during the vasodilatory response; and (3) intramyocardial coronary blood flow appeared to diminish during coronary occlusion and the visualization and measurement of reactive hyperemic coronary flow were obtained by Doppler echocardiography. Using contrast enhancement, these findings could be obtained noninvasively.

The coronary circulation system consists of superficial epicardial coronary arteries, and intramyocardial coronary vessels, such as small coronary arteries, arterioles, capillaries, venules, and through small veins, cardiac veins, coronary sinus, and other structures. The large epicardial vessels course over the left ventricular epicardial surface, giving rise to superficial branches, which subsequently give rise to deep tributaries pen-

etrating the myocardium at a right angle. The tributaries branching from the main epicardial vessels range from 400 to 1,500 μm in diameter. These arteries divide soon after their origin, creating a cascade of branching vessels. Numerous branches supply a large amount of blood to the myocardium, with the caliber of the main perforating vessel gradually diminishing as it extends toward the endocardium (Figure 3).

The epicardial coronary arteries can be imaged by various diagnostic methods, but is mainly imaged by coronary angiogram. Although the epicardial coronary arteries and some small coronary arteries such as "penetrators" are clinically identified by angiographic techniques, intramyocardial coronary arteries are often not visualized nor quantified *in vivo*.¹⁴⁾ The invasive measurement of phasic coronary flow velocity using an intracoronary Doppler guide wire shows alterations in flow velocities distal to severe coronary stenosis.^{15,16)} However, existing information about phasic coronary blood flow by guide wire methods is based primarily on measurements obtained from epicardial coronary arteries which are considered functionally to be capacitors. Kajiya, *et al.* have reported the measurement

Figure 3. Schematic diagram of intramyocardial coronary blood flow.

of intramyocardial blood flow using an invasive laser flow meter in an animal study.¹⁷⁾

Many studies have reported the assessment of epicardial coronary blood flow using transesophageal and most recently transthoracic echocardiography. However, previous applications of transthoracic echocardiography to coronary arteries have been limited to epicardial coronary arteries. The proximal left main coronary artery can be imaged because it is located within the focal zone of the standard imaging frequencies (2.5 to 3.5 MHz).¹⁻³⁾ With the better resolution of transesophageal transducers and an adequate window for the coronary arteries, transesophageal echocardiography is a valuable tool for evaluating epicardial coronary artery flow.^{2,18-20)} High frequency transthoracic echocardiography transducers have been developed and used to detect mid and distal portions of the left anterior descending coronary artery.^{4,21-24)} Recent reports have demonstrated the possibility of noninvasive evaluation of intramyocardial small coronary artery flow in the near transducer field with color Doppler echocardiography.⁸⁻¹⁰⁾ They can assess flow only in fields near the transducer. It has been difficult to image and measure intramyocardial coronary blood flow in all LV wall segments.

Originally, myocardial contrast echocardiography was introduced for the evaluation of myocardial perfusion of coronary microcirculation, which can evaluate capillary artery integrity.²⁵⁾ Intravenous contrast agents can improve the Doppler signal to noise ratio, which can increase the Doppler signal intensity in coronary arteries. Thus, intravenous ultrasonic contrast agents improve the feasibility and quality of Doppler echocardiographic evaluation of coronary blood flow velocity.^{26,27)} We used a lower, rather the generally used, concentration of Levovist solution to reduce artifacts and noise from the microbubble signal in the right ventricle chamber and minimize the Doppler shift phenomenon. In this study, we were able to assess the intramyocardial coronary blood flow of all LV segments using contrast-enhanced color Doppler echocardiography with a high frequency transducer.

In normal epicardial coronary arteries, diastolic flow amounts to approximately three-quarters of the total flow in each cardiac cycle and the velocity of systolic flow is reduced. However, spectral Doppler waveforms of the flow velocity of intramyocardial coronary arteries are almost exclusively diastolic, occasionally accompanied by flow reversal during the isovolumic contraction period.²⁸⁾ The phasic blood flow velocity pattern of intramyocardial coronary arteries is markedly different to that of epicardial arteries.

Our study indicates that the visualization and measurement of intramyocardial coronary blood flow could be obtained by pulsed and color Doppler echocardiography *in vivo*. The intramyocardial coronary arteries can be identified based upon the flow direction of the pulsed Doppler signals directing from the epicardium to endocardium in diastole. Previous studies have reported that the intramyocardial coronary artery is consistently more predominant in diastole than in systole with or without systolic flow reversal.^{29,30} The size of intramyocardial coronaries ranges approximately from 0.4 to 1.5 mm. Using a high frequency transducer, in the black and white images, the axial resolution is about 0.3 to 0.8 mm, and the lateral resolution is 0.8 to 1.1 mm at the depth of 3 cm. In the color Doppler images the axial resolution is about 0.6 to 1.6 mm, and the lateral resolution is about 0.8 to 1.6 mm. Moreover, the resolution is much better in the zooming images than in the standard images. The sample volume (3-5 mm in width) of a pulsed wave Doppler was positioned at the center of the color flow images of the intramyocardial coronary artery. Although vessel diameter is less than 1 mm, the strongest ultrasound signal from the intramyocardial coronary arteries can be measured as a flow velocity by pulsed Doppler echocardiography. Variation in flow velocities due to vasodilatory response could be detected. That is, the vasodilatory response of intramyocardial coronary flow velocity using ATP can be detected by pulsed and color Doppler echocardiography.

In this *in vivo* study, a remarkable decrease in the intramyocardial coronary blood flow velocity of the LV antero-septal region was directly observed after occlusion of the left anterior descending coronary artery by the high frequency ultrasonic transducer of the echocardiogram. Following reperfusion, the coronary blood flow in the LV antero-septal wall gradually reappeared and was subsequently visualized. Reactive hyperemic coronary flow can be observed as a remarkable increase in intensity of color Doppler flow mapping. The flow velocity was also measured using pulsed and color Doppler echocardiography for the first time *in vivo*. Therefore, it is feasible that one can appreciate the reperfused intramyocardial coronary flow by color and pulsed Doppler echocardiography after successful reperfusion of epicardial coronary arteries. Myocardial contrast echocardiography has emerged as a promising imaging tool with which to assess myocardial perfusion. However, as a blood flow tracer, microbubble contrast agent is not perfect and many agents are still in clinical trials. This study was a great step toward direct visual assessment of intramyocardial coronary circulation in patients with ischemic heart disease, specifically differentiating between patients with viable or non-viable myocardium.

The presence of intramyocardial coronary blood flow in the infarcted LV myocardium may suggest a viable myocardium despite severely reduced LV wall motion.

Study Limitations: In this study, intramyocardial coronary blood flow was detected using color Doppler echocardiography with contrast injection in 39 of all 50 segments (78%) in closed chest dogs, and in all segments (100%) in open chest dogs. However, the coronary flow velocities were measured in 30 of 50 segments (60%) in closed chest and in 45 of 50 segments (90%) in open chest. Because of cardiac motion and respiration, it was difficult to record the intramyocardial coronary flow velocity of segments far from the transducer.

Because of an angle of incidence to the intramyocardial coronary arteries of more than 20 degrees, the maximal Doppler flow velocity signal may be slightly underestimated.

We chose not to use angle correction so as not to introduce an additional source of error. In addition, the true peak velocity could not be detected because of the ATP bolus injection. Potentially this yield could be improved by infusion of vasodilators and contrast agents.

As our purpose was to investigate the feasibility of detecting and measuring intramyocardial coronary flow by Doppler echocardiography, we did not compare this technique to other invasive methods.

Conclusions: While few studies for the detection of intramyocardial coronary blood flow by Doppler echocardiography have assessed flow near the transducer field, the findings of this study indicate it is possible to visualize and assess intramyocardial coronary blood flow in the entire left ventricular wall using contrast-enhanced Doppler echocardiography. Intramyocardial coronary blood flow measurements could lead to an improved understanding of coronary circulatory physiology. Though this study was an animal study, we expect the results are applicable in humans.

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