Noninvasive Vessel-Selective Perfusion Imaging with Intravenous Myocardial Contrast Echocardiography

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Abstract

Background: Intravenous myocardial contrast echocardiography (MCE) cannot identify each perfusion area of coronary vessels separately. However, by destroying microbubbles passing through a specific vessel using high-power ultrasound during intravenous MCE, vessel-selective perfusion imaging (VSPI) may be feasible.

Methods: In 10 open-chest dogs, intermittent short-axis images were obtained during Definity infusion using a Sequoia 512 system. For VSPI, an S3 probe coupled to a Sonos 5500 was placed on the proximal left circumflex (LCx) artery. High-power ultrasound pulses were transmitted to destroy bubbles passing through the LCx artery. A negative contrast area on VSPI was considered to represent the perfusion area of the LCx artery (LCx-VSPI). A negative contrast area on conventional MCE during LCx occlusion (LCx-Occ) and a region without staining by Evans blue dye (LCx-EB) were used as gold standards for defining the LCx perfusion area. LCx-VSPI was compared with LCx-Occ and LCx-EB.

Results: Despite lack of LCx artery occlusion, high-power destructive pulses produced a definite area of negative contrast on the LCx region. Decreased power of ultrasound pulses resulted in disappearance of the negative contrast area. An excellent relationship was demonstrated between both LCx-VSPI and LCx-Occ ($r = 0.93$, $P < .0001$), and LCx-VSPI and LCx-EB ($r = 0.92$, $P = .0002$).

Conclusion: VSPI during intravenous MCE may be feasible for noninvasive assessment of perfusion areas associated with specific vessels.

Use of myocardial contrast echocardiography (MCE) was initially limited by the need to inject contrast microbubbles directly into coronary arteries. Recent developments in contrast microbubble technology and ultrasound imaging techniques have permitted assessment of myocardial perfusion using venous injection of microbubbles.(1, 2) Although intravenous MCE represents a promising method for noninvasive myocardial perfusion imaging, some disadvantages are seen compared with intracoronary MCE. For example, intracoronary MCE allows separate identification of the perfusion areas of individual coronary vessels, whereas intravenous MCE does not. Other noninvasive perfusion imaging techniques, like single-photon emission computed tomography, are likewise unable to detect perfusion areas derived from individual vessels.

Microbubbles are known to be susceptible to destruction by sufficient acoustic power.(3,4) We therefore hypothesized that destruction of microbubbles passing through a specific vessel using high-power ultrasound pulses might allow vessel-selective perfusion imaging (VSPI) using intravenous MCE. The present study evaluated whether VSPI can identify the perfusion area for a selected vessel.

Methods

Animal Preparation

All animal studies were performed in accordance with Osaka University Medical School guidelines for the care and use of laboratory animals and American Heart Association guidelines for use of animals in research. A total of 10 open-chest dogs were used in this study. Dogs were anesthetized using intravenous sodium pentobarbital, intubated, and ventilated with room air using a respirator pump. Anesthesia was maintained throughout the experiment, with additional use of anesthetic

as required. An 18-gauge peripheral intravenous catheter was positioned in the foreleg for administration of contrast agent. A 5F catheter was placed in the femoral artery for monitoring blood pressure, and a 7F catheter was placed in the femoral vein for administration of intravenous medications and fluids. Electrocardiography was continuously performed and monitored. Left lateral thoracotomy was undertaken, and the heart was suspended in a pericardial cradle. The proximal portion of the left circumflex (LCx) coronary artery was dissected free from surrounding tissues, and an occluder and flow probe were placed.

MCE

MCE was performed using a Sequoia 512 platform (Acuson/Siemens, Mountain View, Calif) equipped with coherent contrast imaging software in conjunction with a 3V2c transducer transmitting at 1.75 MHz and receiving at 3.5 MHz. Mechanical index (MI) was set at 0.8, and dynamic range at 50 dB. The transducer was maneuvered to yield a short-axis view at the papillary muscle or apical level, then fixed in place using a mechanical arm. Intermittent end-systolic images were acquired at pulsing intervals of 8 cardiac cycles. Gain setting was optimized at the beginning of each experiment. Beam focus was set at the center of the left ventricle. All settings were kept constant throughout each experiment.

Contrast was produced by continuous infusion of Definity (Bristol-Myers Squibb Medical Imaging, Billerica, Mass). A 10% Definity solution was infused intravenously at a rate of 15-45 mL/h to provide optimal myocardial opacification with minimal shadowing of the left ventricular posterior wall. Data were digitally stored on magneto-optical disk.

VSPI

Two transducers were used for VSPI; one for imaging and another for destroying microbubbles passing through a specific vessel. An S3 probe coupled to a Sonos 5500 (Agilent/Philips, Andover, Mass) was gently placed on the proximal LCx artery for bubble destruction. Space between the transducer and artery was filled up with ultrasound transmission gel. Although continuous bubble destruction was considered preferable, destructive ultrasound pulses were scanned at 200-ms intervals to reduce interference of the ultrasound beams from the two transducers. Sector angle was about 45º. Frequency was 1.6 MHz and MI (acoustic power) was set at 1.6 (0 dB), 0.9 (-5 dB), 0.4 (-10 dB), 0.2 (-20 dB), and <0.1 (-30 dB). For imaging, a Sequoia platform and Definity microbubbles were used in the same fashion as conventional MCE. Angle of the transducer for bubble destruction was adjusted so that beams from each transducer would not cross.

Experimental Protocol

First, intermittent short-axis images without contrast were acquired once every cardiac cycle for baseline subtraction. Conventional MCE was performed at a pulsing interval of 8 cardiac cycles, and VSPI was then performed at the same pulsing interval during bubble destruction in the LCx artery. Acoustic power for bubble destruction was varied as mentioned above. The LCx artery was then completely occluded using the occluder, and conventional MCE images were again acquired just after LCx occlusion. In each dog, this sequence was repeated at varying levels of short-axis view. More than 5 intermittent images were recorded for each setting.

At the end of the experiment, the occluder of the LCx artery was removed and the site at which the occluder had been placed was ligated. A 1.5-mL/kg bolus of 5% Evans blue dye was hand-injected into the left atrium after LCx artery ligation. After

dye injection and sacrifice, the heart was excised and cut from apex to base into several transverse slabs of about 10-mm thickness. Slabs were matched with equivalent MCE and VSPI sections. All available anatomic landmarks (e.g., anterior and posterior papillary muscles and right to left ventricular junction) were used to minimize discrepancies in sections studied.

Data Analysis

To investigate optimal acoustic power of destructive ultrasound pulses for VSPI settings, baseline-subtracted myocardial signal intensities were evaluated in the LCx artery area and left anterior descending (LAD) artery area for each MI of destructive pulses using Data Pro software (Acuson/Siemens, Mountain View, Calif). Circular regions of interest were placed in the center of the LCx area and the opposite site at the same depth for the LAD area to measure signal intensities.

Using the optimal VSPI setting thus determined, we subsequently evaluated whether VSPI could assess perfusion area of a specific vessel. A negative contrast area on VSPI was considered to represent perfusion area of the LCx artery (LCx-VSPI). Negative contrast area on MCE during LCx occlusion (LCx-Occ) and region unstained by Evans blue dye (LCx-EB) were used as gold standards for LCx perfusion area, and LCx-VSPI was compared with LCx-Occ and LCx-EB. Values for each were measured using planimetry and expressed as percent of left ventricular myocardium. LCx-VSPI and LCx-Occ values were averaged from 3 images. To avoid underestimation of LCx perfusion area due to collateral flow during coronary occlusion, images were selected from among those acquired immediately after occlusion. Negative contrast areas and anatomical unstained regions were determined by two investigators who were blinded to each other's data.

Statistical Analysis

Data were expressed as mean ± SD. Baseline-subtracted myocardial signal intensities in the LCx and LAD areas at different MIs were compared using repeated-measures ANOVA. Correlations between LCx-VSPI and LCx-Occ, and between LCx-VSPI and LCx-EB, were determined using least-squares fit regression and Bland-Altman analyses. Values of $P < .05$ were considered statistically significant.

Results

In 3 of the 10 dogs, myocardium unstained by Evans blue dye could not be assessed because of under- or overstaining. Another dog displayed idiopathic pulmonary hypertension at the beginning of the study. These 4 dogs were therefore excluded from the analysis.

Optimal Power of Destructive Pulses for VSPI

For deciding optimal acoustic power of destructive pulses for VSPI, 9 short-axis views (6 for papillary muscle level, 3 for apical level) in 6 dogs were analyzed. Despite absence of LCx artery occlusion, high-power destructive pulses produced definite negative contrast areas on the LCx region in all analyzed sections. When power of destructive pulses was decreased, the negative contrast area disappeared (Fig. 1). A comparison of signal intensities during VSPI between LAD and LCx artery areas at different MIs of destructive pulses is shown in Figure 2. Signal intensities in the LCx area were significantly decreased with high-power destructive pulses. Although signal intensities in the LAD area were also decreased with high-power pulses, decrease in the LAD was smaller than that in the LCx. Difference of signal intensities between the LCx and LAD area were significant for MIs of 0.9 (4.3 ± 4.4 vs 11.3 ± 4.8 dB, $P < .05$) and 1.6 (1.6 ± 2.3 vs 8.0 ± 4.4 dB, $P < .05$).

Comparison between Perfusion Area Derived from VSPI and Gold Standards

To evaluate whether VSPI could assess the perfusion area of a specific vessel, MIs of 0.9 or 1.6 were used for destructive pulses in accordance with the above-mentioned results. Ten short-axis views (6 for papillary muscle level, 4 for apical level) from 6 dogs were analyzed for this purpose. A negative contrast area derived from VSPI, a negative contrast area derived from MCE during LCx occlusion, and an unstained region derived from dye in the same dog are shown in Figure 3. The negative contrast area in VSPI was clearly detected and similar to the negative and unstained areas for gold standards. Excellent relationships and agreement were demonstrated between both LCx-VSPI and LCx-Occ ($r = 0.93$, $P < .0001$; Fig. 4), and LCx-VSPI and LCx-EB ($r = 0.92$, $P = .0002$; Fig. 5).

Discussion

The present study introduced VSPI during intravenous MCE and evaluated the feasibility of this novel technique. Our results indicate that high-power destructive ultrasound pulses for VSPI can destroy microbubbles passing through the LCx artery, and the LCx perfusion area derived from VSPI was coincident with the LCx perfusion areas derived from MCE and dye injection during LCx occlusion. The data suggest that the VSPI technique allows noninvasive assessment of the perfusion area of a specific vessel.

Because frequencies of the two transducers were different, we were concerned that destructive pulses might interfere with imaging quality. However, no image deterioration was observed unless two sector scan beams crossed. Although high-power pulses of ultrasound destroyed microbubbles even in perfusion areas of nonselected-vessels (i.e., the LAD area), contrast opacification was enough to delineate

the negative contrast area.

In noninvasive perfusion imaging techniques, including intravenous MCE, specific vessels cannot be selected for examination. These methods are thus unable to identify perfusion area of individual vessels. Intracoronary MCE can separate the perfusion areas of left and right coronary arteries, but the technique is invasive and differentiating between LAD and LCx perfusion is still difficult. In our study, the VSPI technique produced noninvasive visualization of perfusion area for selected vessels, with perfusion area expressed as a negative contrast area. This only represents one noninvasive method for this purpose. Theoretically, the technique will be able to detect the perfusion area of a small branch if destructive pulses are selective enough.

Clinical Implications of VSPI

Assessment of perfusion area for a selected vessel produces valuable information for cardiologists. First, if the jeopardized vessel is known, risk area in case of vessel occlusion can be predicted. Prediction of risk area can influence therapeutic strategies for the jeopardized vessel. Second, the technique may be useful for evaluating bypass graft perfusion. Recently, transthoracic Doppler technique has permitted noninvasive assessment of bypass graft flow. This Doppler method is easily performed and produces information on graft patency, but perfusion area based on the graft is unknown. We have reported that graft perfusion area was recognized as an area of delayed contrast opacification on intravenous MCE.(5) The VSPI technique may also allow evaluation of whether a bypass graft is perfusing the myocardial level. Third, arteries contributing to collateral flow may be detectable using VSPI. Although intravenous MCE can visualize collateral flow in myocardium, this approach cannot identify the donor artery providing collateral flow. In the case of rich collaterals, even if

the coronary artery is occluded, intravenous MCE may demonstrate perfect myocardial opacification. In this case, however, VSPI can identify occlusion of the coronary artery.

VSPI may also prove useful for clinicians other than cardiologists. In patients with tumors, detection of feeding arteries is very important when determining therapeutic strategy. VSPI may be applied to the noninvasive detection of such arteries.

Feasibility of VSPI and Limitations

This experiment represents a first step in developing the VSPI technique. Several issues remain to be addressed before clinical use can be contemplated. An open-chest model was used in the present study, and destructive pulses for VSPI were transmitted directly onto the target vessel. In clinical settings, however, transmission of destructive pulses to a selected vessel may be difficult, as the target vessel needs to be detected using a transthoracic approach. Vessel selection for VSPI may thus be limited. Any potential harmful effects should be also investigated, as acoustic power in the tissue may be increased by the use of two transducers.

Perfusion assessment for bypass grafts using VSPI may represent a more practical application, as the internal mammary artery can be readily found by transthoracic procedure (6,7). Interactions between the two transducers are minimal in such a situation.

Real-time three-dimensional echocardiography has recently become clinically feasible, and allows multi-slice analysis using volume scanning.(8) If the acoustic power of some beams can be selectively increased within this scan, high-power beams would produce discriminative microbubble destruction. With the development of such technology, VSPI may be achievable using only one transducer.

Conclusions

We introduced the VSPI technique with intravenous MCE for noninvasive assessment of the perfusion area derived from a specific vessel. VSPI may be feasible with destruction of microbubbles passing through the target vessel using high-power pulses of ultrasound.

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Figure Legends

Figure 1: Vessel-selective perfusion images with myocardial contrast echocardiography at 2 different acoustic power settings for destroying microbubbles passing through the left circumflex (*LCx*) artery. Despite absence of LCx artery occlusion, high-power destructive pulses produced a definite area of negative contrast on the LCx region (*left*). When power of destructive pulses was decreased, negative contrast area disappeared (*right*).

Figure 2: Comparison of signal intensities on vessel-selective perfusion imaging between left anterior descending (*LAD*) and left circumflex (*LCx*) artery areas for different mechanical indices of destructive pulses. Compared with LAD area * $P < .05$.

Figure 3: A) Vessel-selective perfusion imaging (*VSPI*) with microbubble destruction in the left circumflex (*LCx*) artery. **B)** Myocardial contrast echocardiogrphy (*MCE*) during LCx artery occlusion. **C)** Evans blue dye staining after LCx artery ligation in the same dog. *LCx-VSPI*, LCx area derived from vessel-selective perfusion imaging; *LCx-Occ*, LCx area derived from myocardial contrast echocardiography during LCx artery occlusion; *LCx-EB*, LCx area derived from Evans blue dye after LCx artery ligation.

Figure 4: Regression line and Bland-Altman plot between perfusion areas of the left circumflex (*LCx*) artery derived from vessel-selective perfusion imaging (*LCx-VSPI*) and myocardial contrast echocardiography during LCx artery occlusion (*LCx-Occ*).

Figure 5: Regression line and Bland-Altman plot between perfusion areas of the left circumflex (*LCx*) artery derived from vessel-selective perfusion imaging (*LCx-VSPI*) and Evans blue dye after LCx artery ligation (*LCx-EB*).