Reproducibility of Intravenous Intermittent Triggered Myocardial Contrast Echocardiography in Healthy Subjects

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SUMMARY

Few data have been published on the reproducibility of baseline subtracted peak intensity obtained from intravenous intermittent triggered myocardial contrast echocardiography. We investigated the reproducibility of the peak intensity measured from intravenous intermittent triggered myocardial contrast echocardiography in 10 young healthy males. The contrast echocardiography was obtained using the second harmonic mode with an intravenous bolus injection of Levovist (first study). The same myocardial contrast echocardiography was repeated after the first study (second study). The myocardial opacification and peak intensity in the 12 segments of the apical 4 and 2 chamber views were assessed visually and quantitatively. The differences in the peak intensity between the initial and repeated measurements in the first study (intraobserver reproducibility) and between the initial measurements in the first and second studies (interinjection reproducibility) were assessed using the Bland and Altman method. The degree of opacification was good or intermediate in 207/228 (91%) of the segments. The agreement of myocardial opacification between the first and second studies was 87/114 (76%). However, significantly higher peak intensity was obtained in apical septal (8200 ± 6300 au²) and mid septal (8500 ± 6000 au²) segments in the 4 chamber view and in the mid inferior (12400 ± 9300 au²) and apical inferior (10700 ± 6300 au²) segments in the 2 chamber view compared with other segments. The mean differences of the peak intensities according to the Bland and Altman analysis was -1600 ± 5000 au² in the intraobserver reproducibility study, and -1100 ± 5300 au² in the interinjection reproducibility study. Thus, the measurement error was determined to range from 8400 au² to 9500 au² in both studies. We conclude that the peak intensity obtained from intravenous intermittent triggered myocardial contrast echocardiography using Levovist varies significantly among segments in the left ventricular myocardium. Large intraobserver and interinjection variability exists.

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Received for publication May 15, 2003.
Revised and accepted November 13, 2003.
in the measurement of peak intensity, suggesting that the reproducibility of this technique is limited for quantitative assessment of myocardial perfusion. (Jpn Heart J 2004; 45: 461-473)

**Key words:** Intravenous myocardial contrast echocardiography, Levovist, Reproducibility

**MYOCARDIAL** contrast echocardiography was reported first as a method delineating the myocardium after the intracoronary or aortic root injection of a contrast agent in canine experiments.\(^1\,^2\) This method has also been applied to human clinical studies.\(^3\,^4\) Recently, the development of contrast agent and ultrasonographic equipment technology, such as second harmonic imaging or intermittent triggered imaging, enabled intravenous myocardial contrast echocardiography in both animal experiments\(^1\,^4\) and human studies.\(^5\,^9\) Analysis of the contrast effect in myocardial contrast echocardiograms has been performed by the visual assessment of myocardial opacification\(^1\,^7\,^9\,^10\,^13\) and/or quantitative assessment of pixel intensity.\(^2\,^3\,^6\,^9\,^14\,^15\)

Shapiro, et al\(^16\) reported the reproducibility of quantitative myocardial contrast echocardiography using repeated intracoronary injections of contrast material in an animal study. However, there have been no reports assessing the reproducibility of intravenous myocardial contrast echocardiography. In intermittent harmonic imaging, ultrasound destroys the contrast agent. The interaction of the ultrasound beam with the contrast agent results in nonlinear backscatter among the contrast targets. Nonlinear signals from microbubbles produce a stronger intensity as compared to linear signals from red blood cells and the myocardium. If the microbubbles in the myocardium are distributed uniformly through the blood pool and are destroyed uniformly by ultrasound, the increase in the intensity should be uniform and reproducible, reflecting the blood flow in the myocardium.\(^17\)

However, in a human intravenous intermittent triggered myocardial contrast echocardiographic study, some pitfalls or artifacts, such as displacement of the imaging plane, ultrasound attenuation, extracardiac attenuation by ribs and pulmonary tissue, contrast shadowing, blooming and wall motion artifacts have been noted.\(^18\) If microbubble destruction is not uniform due to these pitfalls or artifacts, the increase in intensity may not be uniform or reproducible. We investigated the reproducibility of increased intensity in the myocardium during intravenous myocardial contrast echocardiography in 10 apparently healthy male subjects.
METHODS

The study group consisted of 10 young healthy Japanese male subjects (mean age, 24 ± 1 years, range, 23 to 27 years). All subjects gave informed consent to participate in the study and the protocol was approved by the review board of our hospital.

**Contrast agent:** The ultrasound contrast agent used was Levovist (Schering and Tanabe Seiyaku, Tokyo), a first generation contrast agent composed of semifree air - filled microbubbles stabilized by palmitic acid in a galactose - water solution.19,20 The agent was prepared 2 minutes before injection by suspending 2.5 g of galactose microparticles in 7 mL sterile water to obtain an effective concentration of 300 mg/mL. An intravenous line was established using a 20–gauge catheter in the right antecubital vein. The intravenous bolus injection of 3 mL of Levovist was performed manually at a rate of 0.5 mL/s.

**Two–dimensional echocardiography:** Transthoracic 2-dimensional echocardiography was performed with subjects in the left lateral supine position and during quiet expiration. A commercially available phased array system (Philips Medical Systems SONOS 5500, Andover, MA, USA) was used. Intermittent second harmonic imaging was performed using transmission and receipt frequencies of 1.8 and 3.6 MHz, respectively. The triggering interval was set at once per 4 cardiac cycles, at end systole. A mechanical index of 1.6 was set at maximum and the gain settings were optimized at the onset of each study and held constant throughout. The focus was set at the mid left ventricular cavity. The myocardial contrast echocardiographic plane of the apical 4 chamber view was kept constant after bolus injection of Levovist and stored digitally on the echocardiographic system. The images were down-loaded on-line to a removable 5 inch magnetic/optical disk (Figure 1). After complete disappearance of the initially injected Levovist from the left ventricle, the myocardial contrast echocardiographic study for the apical 2 chamber view was performed by the same method and same investigator (first study). The same myocardiographic contrast echocardiographic studies for apical 4 and 2 chamber views were then repeated again using another bolus injection of Levovist (second study). Each subject received 4 bolus injection using 2 vials of Levovist. The time interval between each injection was 10 minutes according to the product information from the company.

**Image analysis:** The left ventricular myocardium was divided into 12 segments using the apical 4 and 2 chamber views according to the American Society of Echocardiography recommendation. The degree of opacification of each segment after administration of the contrast agent was graded visually as good opacification, intermediate opacification, or poor or no opacification visible. Furthermore, quantitative analysis of the intensity was used to objectively evaluate the ultrasonographic contrast effect in the left ventricular myocardium. A personal com-
A computer equipped with an optical disk drive was used to read the images and the data were transferred to an EchoTech 3D Imaging System (QuantiCon, Munich, Germany). The circular regions of interest (ROIs) from which the data were acquired were located in the center of each basal, mid, and apical left ventricular segment. Care was taken to exclude cardiac structures such as the endocardium or papillary muscle. In the myocardium, the endocardial and epicardial borders were carefully avoided. Frame-to-frame drift as well as translation and motion due to respiration in the cross-sectional image were manually corrected using motion-correction software installed in the system. The mean pixel value of an ROI was computed frame by frame using acoustic densitometry, and time intensity curves were generated (Figure 2). Acoustic densitometry provided an integrated capability to

Figure 1. Examples of intravenous myocardial contrast echocardiography of the apical 4 chamber view. Echocardiograms before contrast agent injection (panel A), at right ventricular opacification (panel B), at left ventricular opacification (panel C), and at left ventricular myocardial opacification (panel D) are presented. LA = left atrium; LV = left ventricle; RA = right atrium; RV = right ventricle.
measure, display, and analyze the average acoustic image intensity within the ROI. We chose to display the ultrasound image data measurement in intensity units obtained by squaring the voltage to span a range from 0 to 65536 (au² or V²).

To assess the time intervals from the injection of Levovist to myocardial opacification, the number of frames from the injection to the beginning of the intensity increase and to the peak intensity were obtained from the time-intensity curve. To correct for background intensity, the baseline-subtracted peak intensity was determined by subtracting the baseline intensity, as determined from the end-systolic frames preceding the appearance of contrast, from the peak intensity. Changes in intensity were only considered if a time-intensity curve could be clearly discerned, and if the peak intensity of this curve exceeded the standard deviation of the respective background value.9)

**Reproducibility analysis:** To assess the reproducibility of intravenous bolus myocardial contrast echocardiography, we compared the heart rate and number of echocardiographic frames from the injection to the beginning of the intensity increase and to the peak intensity obtained from the time-intensity curve between the first and second studies. We also visually compared the echocardiographic plane and the

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**Figure 2.** Measurement of myocardial intensity in the apical 4 chamber view using a QuantiCon. Myocardial intensity was measured in the circular regions of interest (a, b, c, d, e and f) located in the center of 6 segments. The right panel shows the time intensity curve in each region of interest. LA = left atrium; LV = left ventricle; RA = right atrium; RV = right ventricle.
degree of myocardial opacification between the first and second studies. To assess intraobserver reproducibility in the measurement of baseline-subtracted peak intensity, the peak intensity was quantified in 90 segments of the first contrast study at a different time by a single observer. The differences in peak intensity between the initial and repeated measurements in the first study were assessed. To assess the interinjection reproducibility of the peak intensity, we compared the peak intensity in 114 segments between the initial measurements in the first study and the second study. The same reproducibility analysis was performed in segments showing good opacification \((n = 46)\) and intermediate opacification \((n = 36)\) in which agreement of visual image analysis was obtained between the first and second studies.

**Statistical analysis:** Values are expressed as the mean ± SD. The differences between two groups were assessed by the paired Student \(t\) test. Differences among groups were assessed by one-way analysis of variance, followed by an appropriate multiple comparison procedure. Intraobserver reproducibility and interinjection reproducibility in the measurement of the baseline-subtracted peak intensity were assessed using the Bland and Altman analysis.\(^{21}\) Statistical significance was defined as \(P < 0.05\).

**RESULTS**

Both the first and second studies were successfully conducted in all 10 subjects. However, image digitization failed in 1 subject in the apical 2 chamber study. Therefore, 60 segments in 10 subjects were analyzed in the apical 4 chamber view study, and 54 segments in 9 subjects were analyzed in the apical 2 chamber view study.

**Heart rate and number of frames to the intensity increase and peak intensity:** There were no significant differences in the heart rate between the first and second studies of the apical 4 and 2 chamber views nor any significant differences between the first and second studies in the number of frames from the injection of Levovist to the beginning of the intensity increase. There was no significant difference between the first and second studies for the 4 chamber view in the number of frames until peak intensity. However, the number of frames in the second study for the 2 chamber view was significantly increased compared to that in the first study (Table I).

**Visual image analysis:** Visually equivalent echocardiographic planes of apical 4 and 2 chamber views were obtained between the first and second studies for all studies. The degree of opacification was good or intermediate in 207/228 (91%) of the segments for the apical 4 chamber and 2 chamber views (Table II). The agreement rate of the opacification between the first and second studies was 45/
60 (75%) in the segments for the 4 chamber view, 42/54 (78%) in the segments for the 2 chamber view, and 87/114 (76%) in all segments.

**Quantitative intensity analysis:** All intensity measurements were below 65536 au². The baseline-subtracted peak intensity was 9000 ± 7000 au² in the segments showing good visual opacification and 3300 ± 3500 au² in the segments of intermediate visual opacification. The peak intensity in the segments for apical 4 chamber view were as follows. The peak intensity in the apical septum (8200 ± 6300 au²) and mid septum (8500 ± 6000 au²) was significantly higher than in the basal lateral (800 ± 1200 au²), mid lateral (3900 ± 4600 au²), and basal septal (2300 ± 2300 au²) segments. The peak intensity in the apical lateral (5900 ± 4800 au²) segment was significantly higher than in the basal lateral and basal septal segments. The peak intensity in the mid lateral segment was significantly increased compared to the basal lateral segment. The peak intensity in the mid inferior (12400 ± 9300 au²) and apical inferior (10700 ± 6300 au²) segments of the apical 2 chamber view were significantly increased compared to the basal inferior (4800 ± 4300 au²), apical anterior (3100 ± 3400 au²), mid anterior (2700 ± 5700 au²), and basal anterior (2100 ± 5400 au²) segments.
Intraobserver and interinjection reproducibility studies: Significant correlation of the baseline-subtracted peak intensity was observed between the initial and repeated measurements of the first study ($r = 0.78$, $n = 90$, $P < 0.001$). The differences in the peak intensities according to the Bland and Altman analysis were $-1600 \pm 5000 \text{ au}^2$ (Figure 3). Significant correlation of the intensity was also observed between the initial measurements of the first and second studies ($r = 0.66$, $n = 114$, $P < 0.001$). The differences in the peak intensities increased gradually as the mean value of the peak intensities increased and the differences were $-1100 \pm 5300 \text{ au}^2$ (Figure 4). Thus, the measurement errors according to Bland and Altman in the intraobserver and interinjection studies ranged from 8400 au$^2$ to 9500 au$^2$.

Figure 3. Regression (left) and difference (right) plots in the intraobserver reproducibility study, comparing the peak intensity obtained from the initial and repeated measurements in the first study. PI = baseline-subtracted peak intensity.

Figure 4. Regression (left) and difference (right) plots in the interinjection reproducibility study, comparing the peak intensity from the initial measurements in the first and second studies. The differences in peak intensity increased as the mean value of peak intensity increased. PI = baseline-subtracted peak intensity.
A positive correlation for peak intensity was also observed between segments showing good opacification in the first and second studies ($r = 0.65$, $n = 46$, $P < 0.001$). However, the difference in intensities was $-1400 \pm 5900$ au$^2$. There was no correlation in peak intensity between segments showing intermediate opacification in the first and second studies ($n = 36$).

**DISCUSSION**

In this intravenous myocardial contrast echocardiographic study, we showed that 1) the baseline-subtracted peak intensity was significantly different among segments in the myocardium of normal subjects, and 2) intraobserver and interinjection reproducibility studies in the measurement of the peak intensity showed that the measurement error was large in both studies, in spite of the good agreement for myocardial opacification. Measurement errors from 8400 au$^2$ to 9500 au$^2$ almost equalled the mean value of the baseline-subtracted peak intensity in the apical and mid septum, which showed a higher peak intensity than other segments in the apical 4 chamber view.

**Variability of the peak intensity in the myocardium:** Before discussing the reproducibility of myocardial contrast echocardiography, we will discuss the variability of the baseline-subtracted peak intensity among segments in the myocardium of normal subjects. Our data suggest that microbubble destruction in the echocardiographic plane was not uniform in our normal subjects, who would be expected to demonstrate uniform myocardial blood flow among the myocardial segments. The reason for nonuniform destruction of the microbubbles is likely multifactorial. Ultrasound energy attenuates as it passes through tissue, thus leaving less energy to destroy the microbubbles in areas farther away from the transducer. Microbubbles in the peripheral parts of the ultrasound field are exposed to less energy, resulting in less destruction in these regions.

Porter, *et al* measured the spatial extent of perfusion defects in dogs with total occlusion of the left anterior descending coronary artery. They showed that the area of the perfusion defect varied significantly according to the distance between the risk area and the transducer. Kolias, *et al* also measured the percent decrease in the video intensity of the destruction frame in 10 patients with no previous history of myocardial infarction using triggered sequential dual-frame power Doppler imaging. The percent decrease in video intensity varied significantly by myocardial location with greater destruction seen in the apical than in the basal regions. These results strongly suggest that ultrasound beam attenuation plays an important role when contrast defects or video intensity are either visually or quantitatively assessed using intermittent harmonic imaging.
In a phased array transducer, steering reduces the intensity of the ultrasound beam so that ultrasound pressure is less at the edges of the sector.\textsuperscript{26} Thus, microbubble destruction may be less at the lateral portion of the echocardiographic plane compared to the central portion of the plane.

**Reproducibility of intravenous myocardial contrast echocardiography:** Many factors, such as heart rate, blood pressure, bubble concentration, injection method, or echocardiographic plane, may affect the reproducibility of intravenous myocardial contrast echocardiography. We showed the reproducibility of heart rate and time interval from the bolus injection of Levovist to the beginning of the intensity increase in this study. However, we did not assess the reproducibility of blood pressure or bubble concentrations before and after bolus injections.

Continuous infusion of microbubbles may provide more consistent bubble administration than bolus injection. It is clinically accepted that a constant Levovist bubble concentration persists for at least 8 minutes during continuous infusion.\textsuperscript{19} Compared to continuous infusion, bolus injection is an easy and simple method in a clinical study.\textsuperscript{20} In our study, bolus injection was performed at the same time for the same echocardiographic view after the preparation of Levovist in the first and second studies. We showed the reproducibility of visually equivalent echocardiographic planes on apical 4 and 2 chamber views, although we did not assess quantitative changes in the plane.

**Intraobserver reproducibility:** In this study, the observer evaluated the intensity in the center of the left ventricular segments. If the ROI was positioned at the same site during both initial and repeated measurements in the first study, the intensity should be the same in both measurements. Large variability in the intensity suggests that the ROI was not positioned at the same site, although the site of ROI must be close between the measurements. Therefore, large measurement variability in the intraobserver reproducibility study suggests that myocardial peak intensity varies in the neighbouring myocardium.

Regional heterogeneity on myocardial contrast echocardiography, observed as a perfusion defect in patients without myocardial infarction, has been reported.\textsuperscript{27} Heterogeneity of the contrast effect during intermittent second harmonic myocardial contrast echocardiography was also observed in healthy patients.\textsuperscript{28} Left ventricular high signal intensity may cause shadowing\textsuperscript{18} or blooming\textsuperscript{29} of the signal, resulting in variable intensity in the myocardium. Contrast signals which originate from intramyocardial small vessels may spread into the neighbouring myocardium due to blooming phenomenon.\textsuperscript{18} If the area of ROI is too small and contains such vessels, the contrast signal intensity may be higher than the neighbouring myocardium even in normal subjects. Therefore, the size
of the ROI used for measuring the intensity may be an important factor influencing the variability of peak intensity.

The same reasons for measurement variability were thought to explain why a large difference in peak intensity was observed in segments showing good opacification in which agreement for visual analysis was obtained between the first and second studies.

**Interinjection reproducibility:** In triggered imaging, the ultrasound transducer must be fixed blindly throughout the study. Small displacement of the imaging plane, that could not be found on visual analysis, may occur due to breathing or displacement of the scan head, resulting in the nonuniform destruction of microbubbles due to the change in ultrasound energy. Nonuniform destruction may also be caused by extracardiac attenuation by the ribs or pulmonary tissue, or wall motion artifacts.

Janerot-Sjöberg, et al.\(^{30}\) found that myocardial signal intensity varies significantly with respiration. Intrathoracic respiratory movement of the heart may cause a change in ultrasound energy during destruction of the microbubbles, resulting in an increased intensity variability or a different time interval until peak intensity between the first and second studies on 2 chamber view. The effect of respiration on the intensity variability seems to be large, because it is impossible to perform intravenous intermittent myocardial contrast echocardiography during end-expiratory apnea in a human clinical study.

As discussed in the intraobserver variability study, technical limitation of the observer in measuring the intensity at the same site in the first and second studies may cause further variability in interinjection reproducibility.

**Acoustic densitometry:** Densitometric analysis has been performed routinely in off-line systems using video-taped images. Kaul\(^{31}\) noted that for the purposes of signal quantification, standard video intensity measurements are not reliable because the relationship between microbubble concentration and video intensity is nonlinear. The nonlinear portion of the curve is reached at smaller microbubble concentrations when log compression and postprocessing is used. In comparison, when the signal is measured prior to manipulations such as acoustic densitometry, the relation becomes nonlinear at much higher concentrations. Therefore, we used acoustic densitometry in the measurement of myocardial intensity. We chose to display the ultrasound image data in intensity units obtained by squaring the voltage (V-SQUARE or au\(^2\)) to span a range from 0 to 65,536. This particular scale not only linearized the ultrasound instrument by correcting the compression and postprocessing sources of nonlinearity, but also linearized the response of the instrument to the concentration of contrast microbubble scatters. As we used a more sensitive and accurate method than videodensitometry, our data may have widespread measurements.
Conclusions: Peak intensity obtained by intravenous intermittent triggered myocardial contrast echocardiography varies significantly among segments in the left ventricular myocardium in normal subjects, in spite of the good agreement of myocardial opacification in the visual assessment. Large intraobserver and inter-injection variability exist in the measurement of peak intensity, suggesting that the reproducibility of intravenous intermittent triggered myocardial contrast echocardiography using Levovist is limited for the quantitative assessment of myocardial perfusion. This nonuniformity of intensity needs to be taken into account when evaluating the clinical application of myocardial perfusion assessment using this technique.

REFERENCES


