Destruction of Microbubbles in the Ventricular Cavities Affects the Quantitative Assessment of Myocardial Perfusion During Real-time Myocardial Contrast Echocardiography

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Abstract

Background. Myocardial perfusion can be measured by assessing the replenishment of bubbles destroyed by transient high power ultrasound waves (burst) during real-time myocardial contrast echocardiography (MCE). However, the burst procedure may destroy microbubbles in ventricular cavities as well as in the myocardium, which can interfere with the accurate measurement of myocardial perfusion. The goal of the present study was to determine the effect of burst exposure to the ventricular cavities on the replenishment curve parameters during real-time MCE.

Methods. The myocardial opacification of the left ventricular (LV) short-axis view was observed using SIEMENS Sequoia-512 (mechanical index = 0.1) during infusion of Optison in 11 open-chest dogs. A 1-second or 6-second ultrasound burst was applied to the right ventricle (RV-burst), lateral wall (Myo-burst), or in the usual fashion (General-burst). The LV cavity was insonified by the General-burst but not by the RV-burst or Myo-burst. The time course of myocardial opacification of the lateral wall after burst was fitted to an exponential function: $y=a(1-e^{-\beta t})+c$.

Results. Although the opacification inside the RV cavity recovered soon after RV-burst regardless of burst duration (1-second burst, 0.88 ± 0.42 sec; 6-second burst, 0.82 ± 0.37 sec), the recovery time of opacification inside the LV cavity was significantly prolonged after the 6-second RV-burst (1-second burst, 0.49 ± 0.39 sec; 6-second burst, 4.30 ± 1.14 sec). Further, in the General-burst, the β -value was significantly lower with the 6-second burst than with the 1-second burst (1-second burst, 0.29 ± 0.10 vs. 6-second burst, 0.11 ± 0.05). However, with the Myo-burst, the β -value was similar when comparing the two burst durations (1-second burst, 0.38 ± 0.13 vs. 6-second burst, 0.35 ± 0.09).

Conclusions. In real-time MCE, the replenishment curve was significantly influenced by the burst procedure area, possibly due to bubble destruction in the ventricular cavities. However, this effect was minimized by the use of a short burst duration.

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Key words: ultrasound, contrast echocardiography, coronary microcirculation

Introduction

Real-time myocardial contrast echocardiography (MCE) can be used to assess myocardial perfusion and to characterize wall motion abnormalities. This quanti-

tative assessment is based on the replenishment of destroyed microbubbles within the circulation [1], and myocardial blood volume and blood flow speed of the microcirculation can be derived from this measurement [2-8]. However, the transient high power ultrasound waves (burst) used to destroy microbubbles in the myocardial microcirculation may also destroy the replenishing bubbles in the ventricular cavities and thereby result in inaccurate measurements of myocardial perfusion. Therefore, the goal of the present study was to determine the effect of burst exposure to the ventricular cavities on the replenishment curve parameters during real-time MCE.

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Methods

Animal preparation

Eleven healthy dogs (13.8 ± 0.8 kg) were anesthetized with a bolus of intravenous pentobarbital sodium (35 mg/kg) followed by a continuous infusion (6-8 mg/kg/hr). Animals were intubated with an endotracheal tube and ventilated with a Harvard-type respirator (Model SN-480-3, Shinano Manufacturing Co. Ltd., Tokyo, Japan) using room air. A left lateral thoracotomy was performed at the fifth intercostal space, and the heart was suspended in a pericardial cradle. An acoustic interface (Sonar-AID, Geistlich-Pharma AG, Wolhusen, Switzerland) was set between the transducer and the anterior surface of the heart. The transducer was fixed along the left ventricular (LV) short-axis view plane at the mid-papillary muscle level with a hand-made clamp.

All protocols were approved by the Osaka University Medical School Animal Care and Use Committee and were in compliance with the Osaka University Medical School guidelines for the care and use of laboratory animals.

Real-time MCE

Optison (GE Amersham Health, Oslo, Norway) was administered continuously (0.1-0.2 ml/min) via a catheter placed in the forearm. Myocardial opacification was observed using real-time [mechanical index (MI) = 0.1; frame rate, 20 Hz] coherent contrast imaging of Sequoia-512 (SIEMENS Medical Solutions USA

Inc., Mountain View, CA) with a 3V2c transducer. Coherent contrast imaging (dynamic range = 50 dB) was performed with a transmitted frequency of 1.75 MHz and a received frequency of 3.5 MHz. The overall gain setting and image depth were optimized at the beginning of the experiment and kept constant throughout the experiment. The position of focus was set at the center of the LV cavity. During the examination of the effect of burst on the replenishment curve, respiration was temporarily held to fix the two-dimensional section plane and to avoid respiratory variation in the heart rate. All images were recorded on videotape with a high-fidelity S-VHS recorder (SVO-9600, SONY Co., Tokyo, Japan).

Setting of burst area and its duration

As the Sequoia-512 apparatus allows independent designation of the acoustic power of two-dimensional echocardiography and color Doppler imaging, and the angle and direction of color Doppler imaging can be set arbitrarily, the burst area was controlled with the color Doppler mode. The MI of the burst procedure was set as maximal (1.8 to 1.9). Three different burst areas were employed in this study: 1) the right ventricular (RV) cavity (RV-burst), to examine the influence of the bubbles inside the RV and LV cavities, 2) the LV lateral wall (Myo-burst), to eliminate any influence of bubble destruction inside the cardiac cavities, and 3) the whole acoustic field area, in which the conventional burst procedure was used (General-burst) (Figure 1).

Fig. 1. Burst area. While the usual burst procedure covers the whole acoustic field (General-burst, right panel), the area of burst can be narrowed and localized [RV cavity (RV-burst, left panel) or the lateral wall (Myo-burst, middle panel)] using the color Doppler mode. The actual area of bubble destruction by the burst procedure was slightly wider than the region of burst, due to the spread of ultrasound. *Yellow frames*: regions of burst, *Orange circles*: regions of interest.

To examine the effect of burst duration, short (1-second; approximately 20 frames) and long (6-second; approximately 140 frames) burst durations were employed for the localized and general bursts.

(1) RV-burst

Opacification of the RV and LV cavities was examined in 5 dogs undergoing RV-burst. Consecutive end-diastolic images before and after the burst procedure were imported to the Color Cardiology Work Station (Nihon Kohden Co., Tokyo, Japan). Circular regions of interest (ROIs) of approximately 1.2 cm in diameter were drawn onto the RV and LV cavity images, and the video intensity of their opacification was measured in grey scale (256 levels) (Figure 1, left panel). The RV and LV opacification time for recovery to 90% of before-burst video intensity was calculated (90% recovery time).

(2) Myo-burst and General-burst

Myocardial opacification was examined in 6 dogs undergoing Myo-burst and General-burst. Consecutive real-time images (150 frames) before and after the burst procedure were digitally captured, and the video intensity of the myocardial opacification was measured

using decibel (dB) units by an off-line computer (Data Pro, Noesis, Courtaboeuf, France). An ROI of approximately 1.0 cm in diameter was placed onto the lateral wall images (Figure 1 middle and right panel), with subsequent manual adjustment of the ROI position to correct for the translational and rotational motion of the heart. The temporal change of video intensity after the burst procedure was fitted to an exponential function: $y=a(1-e^{-\beta t})+c$ using commercially available software (Origin6.0j, Microcal, North Hampton, MA) [7]. The time after the burst procedure was designated as the t-value; the video intensity at time t was designated as the y-value; and the video intensity just after the burst procedure was designated as the c-value. In this study, the averaged video intensity before the burst procedure was calculated and designated as the plateau video intensity, which was calculated as the sum of a and c.

Statistical analysis

Data are represented as mean $\pm$ SD. Statistical analysis of comparisons of 90% recovery time of RV and LV opacification between the short and long duration bursts were performed using the paired t-test. Further, analysis of variance (ANOVA) with the post-hoc Bonferroni test was used to compare myocardial perfusion parameters. A two-sided P value less than 0.05 was considered as statically significant for all comparisons.

Fig. 2. Temporal changes of RV and LV opacification after RV-burst. A long duration burst results in delayed recovery of LV opacification. However, RV cavity opacification recovered soon after the termination of the RV-burst irrespective of the burst duration. (RV, right ventricular; LV, left ventricular)

Fig. 3. The 90% recovery time of RV and LV opacification after the RV-burst. The recovery time of RV opacification was similar when comparing the short and long burst durations. However, the recovery time of the LV opacification was significantly prolonged with the long duration burst when compared with the short duration burst.

*: $p < 0.05$ vs. short burst.

(RV, right ventricular; LV, left ventricular)

Results

(1) Influence of RV-burst on the opacification of the RV and LV cavities

Temporal changes of the opacification of the RV and LV cavities after RV-burst are shown in Figure 2. The 90% recovery time of RV opacification was similar when comparing the short and long duration burst (short, 0.69 sec vs. long, 0.96 sec). However, the 90% recovery time of LV opacification was delayed with the long

duration burst when compared with the short duration burst (short, 0.14 sec vs. long, 5.37 sec).

The 90% recovery time of RV and LV opacification calculated from all subjects is shown in Figure 3. Although the recovery time of RV opacification remained constant irrespective of the burst duration (short, 0.88 ± 0.42 sec vs. long, 0.82 ± 0.37 sec), the recovery time of LV opacification was significantly longer after the long duration burst when compared with the short duration burst (short, 0.49 ± 0.39 sec vs.

Fig. 4. Temporal changes of video intensity of the lateral wall after General-burst (left panel) and Myo-burst (right panel). In the General-burst, the increase in video intensity after burst procedure was attenuated with the long duration burst when compared with the short duration burst. By contrast, in the Myo-burst, the increase in video intensity after the burst procedure was similar when comparing the long and short burst durations.

Fig. 5. Parameters of the myocardial perfusion. The video intensity before the burst procedure (plateau video intensity) was generally similar when comparing the experimental groups (left panel). However, the β -value was significantly reduced after the long General-burst when compared with other groups (right panel). *: $p < 0.05$ vs. other groups.

long, 4.30 ± 1.14 sec).

(2) Effect of General-burst and Myo-burst on myocardial perfusion parameters

Representative cases of the replenishment curves derived after the General-burst and Myo-burst are shown in Figure 4. In the General-burst, the upstroke of the replenishment curve was attenuated with the long duration burst when compared with the short duration burst (Figure 4, left). By contrast, in the Myo-burst, the upstroke of the replenishment curve remained constant regardless of the burst duration (Figure 4, right).

The video intensities of the lateral wall before the burst procedure were generally similar among the experimental groups (General-burst: short, 16.4 ± 2.3 dB vs. long, 17.3 ± 3.3 dB; Myo-burst: short, 16.9 ± 2.5 dB vs. long, 16.8 ± 2.1 dB) (Figure 5, left). However, the β -values derived after the long duration burst were significantly lower with the General-burst than with the Myo-burst (General-burst: short, 0.29 ± 0.10 vs. long, 0.11 ± 0.05 ; Myo-burst: short, 0.38 ± 0.13 vs. long, 0.35 ± 0.09) (Figure 5, right). The β -values derived after the short duration burst were similar when comparing the General-burst to the Myo-burst.

Discussion

The burst procedure is a critical procedural aspect of the replenishment curve determination of myocardial opacification in real-time MCE that reflects myocardial perfusion. However, the high acoustic power used for the burst procedure destroys bubbles in the ventricular cavities as well as in the myocardium, which can result in inaccurate measurements of myocardial perfusion. The present study demonstrated that microbubbles in the ventricular cavities were indeed destroyed by the high power ultrasound exposure. Specifically, a portion of the microbubbles in the RV cavity was destroyed by the conventional longer duration burst procedure, resulting in attenuation of opacification of the LV cavity (Figure 3). However, this effect was minimized by the use of a shorter burst duration.

The present study also demonstrated that the replenishment curves derived after the Myo-burst were similar when comparing the long and short duration bursts (Figures 4 and 5). Furthermore, even if the bubbles in the LV and RV cavities were destroyed by the conventional burst procedure (General-burst), the β -value remained unchanged with the short burst duration. Indeed, the β -value with the short duration

burst was similar when comparing the General-burst and the Myo-burst (Figure 5).

Leong-Poi *et al.* demonstrated the effect of excess bubble destruction inside the LV cavity on parameters of the replenishment curve by the use of short-axis and modified axial planes in real-time MCE [9]. There are two major study design differences between theirs and ours; one is how to destroy microbubbles. Although Leong-Poi *et al.* avoided the destruction of bubbles inside the LV cavity by the use of a modified axial plane, we employed the color Doppler method for bubble destruction. Thereby, we could assess the effect of bubble destruction inside the ventricular cavities on replenishment curve parameters by only the single short-axis plane. The other is how to fit an exponential function to the myocardial opacification time course. Leong-Poi obtained a replenishment curve only with the every end-systolic images after the burst procedure; in this study the replenishment curve was fitted to the consecutive data after the burst procedure. Additionally, the number of images that we could capture was limited to 150 frames. Therefore, a replenishment curve could not be fitted correctly in some subjects with the insufficient rise of video intensity after the burst procedure. To overcome this point, the averaged video intensity before the burst procedure was utilized as the plateau video intensity of a replenishment curve. By the replenishment curve analysis, the use of video intensity before the burst procedure as the plateau intensity would be scarce. However, our results are very consistent with theirs [9]. Thus, the consistency of results would support the validity of an alternative use of video intensity before the burst procedure in our study.

Ventricular cavity microbubble destruction appears to be inevitable when using the General-burst, even with a short burst duration in real-time MCE. By contrast, an overly short burst duration might result in incomplete destruction of bubbles inside the myocardium. Indeed, Bahlmann *et al.* reported that the microbubbles were not completely destroyed even after several high intensity ultrasound pulses in real-time MCE [10]. We reported that incomplete bubble destruction results in a different β -value of the replenishment curve [11]. These data suggest that an extremely short burst would be unsuitable for the quantitative assessment of myocardial perfusion by a replenishment curve in real-time MCE.

Additionally, the destruction of microbubbles inside

the ventricular cavities might be induced in the intermittent triggered imaging also, especially with the short triggering intervals. Leong-Poi et al reported that the destruction of microbubbles inside the LV cavity did not influence the β -value when they employed the pulsing interval longer than every one cardiac cycle [9]. However, it is still unclear whether the β -value is affected by the destruction of microbubbles inside the ventricular cavities with the pulsing interval shorter than every one cardiac cycle. Further studies may be needed to elucidate this issue.

The present study demonstrated one of the potential techniques to quantify myocardial perfusion without excess destruction of microbubbles inside ventricular cavities in real-time MCE. The localized burst would be feasible by the use of color Doppler mode, and the destruction of microbubbles inside the ventricular cavities would be negligible. However, an alternative method to quantify myocardial perfusion more easily and more robust may appear in the future.

Limitations

Steady density of microbubbles in the LV cavity is critical for the accurate calculation of the β -value, as LV cavity microbubbles serve as the immediate resource for replenishment of myocardial microbubbles. However, the present study did not investigate the effect of the localized burst on the LV cavity, because the inflow trunk of the RV cavity was positioned behind the LV cavity in our experiment. To examine the effect of diminished microbubbles inside the LV cavity on myocardial perfusion parameters, the apical approach is likely required, as suggested by Leong-Poi et al. [9].

Conclusions

In real-time MCE, the burst procedure destroys microbubbles in the ventricular cavities, resulting in inaccurate measurements of myocardial perfusion. However, this effect can be minimized by the use of a short burst duration.

References

1. Wei K, Jayaweera AR, Firoozan S, Linka A, Skyba DM, Kaul S. Quantification of myocardial blood flow with ultrasound-induced destruction of microbubbles administered as a constant venous infusion. *Circulation*. 1998; 97: 473-83.
2. Masugata H, Lafitte S, Peters B, Strachan GM, DeMaria AN. Comparison of real-time and intermittent triggered myocardial contrast echocardiography for quantification of coronary stenosis severity and transmural perfusion gradient. *Circulation*. 2001; 104: 1550-6.
3. Masugata H, Peters B, Lafitte S, Strachan GM, Ohmori K, DeMaria AN. Quantitative assessment of myocardial perfusion during graded coronary stenosis by real-time myocardial contrast echo refilling curves. *J Am Coll Cardiol* 2001; 37: 262-9.
4. Leong-Poi H, Le E, Rim SJ, Sakuma T, Kaul S, Wei K. Quantification of myocardial perfusion and determination of coronary stenosis severity during hyperemia using real-time myocardial contrast echocardiography. *J Am Soc Echocardiogr* 2001; 14: 1173-82.
5. Lafitte S, Masugata H, Peters B, et al. Comparative value of dobutamine and adenosine stress in the detection of coronary stenosis with myocardial contrast echocardiography. *Circulation*. 2001; 103: 2724-30.
6. Camp GV, Ay T, Pasquet A, et al. Quantification of myocardial blood flow and assessment of its transmural distribution with real-time power modulation myocardial contrast echocardiography. *J Am Soc Echocardiogr* 2003; 16: 263-70.
7. Otani K, Toshida T, Iwata A, Asanuma T, Ishikura F, Beppu S. Adenosine triphosphate stress myocardial contrast echocardiography detects coronary artery stenosis with greater sensitivity than wall-motion abnormality measurements. *J Am Soc Echocardiogr* 2004; 17: 1275-80.
8. Iwata A, Ishikura F, Otani K, Toshida T, Asanuma T, Beppu S. Nicorandil preserves coronary collateral circulation compared to nitroglycerin even in systemic hypotension: a real-time myocardial contrast echocardiographic study. *J Echocardiogr* 2004; 2: 90-8.
9. Leong-Poi H, Swales J, Jayaweera AR, Bin JP, Kaul S, Lindner JR. Effect of microbubbles exposure to ultrasound on quantitation of myocardial perfusion. *Echocardiography* 2005; 22: 503-9.
10. Bahlmann EB, McQuillan BM, Handschumacher MD, et al. Effect of destructive pulse duration on the detection of myocardial perfusion in myocardial contrast echocardiography: in vitro and in vivo observations. *J Am Soc Echocardiogr* 2002; 15: 1440-7.
11. Otani K, Masuda K, Asanuma T, et al. Incomplete bubble destruction affects quantitative assessment of myocardial perfusion in real-time myocardial contrast echocardiography. *J Echocardiogr* 2005; 3: 21-6.