Dynamics of flow velocities in endocardial and epicardial coronary arterioles

Eiji Toyota,1 Yasuo Ogasawara,1 Osamu Hiramatsu,1 Hiroyuki Tachibana,1 Fumihiko Kajiya,1,2 Shinji Yamamori,3 and William M. Chilian4

1Department of Medical Engineering and Systems Cardiology, Kawasaki Medical School, Kurashiki, Okayama; 2Department of Physiology II, Okayama University Medical School, Shikatcha-cho, Okayama-shi, Okayama; 3Sensor Division, Engineering Operations, Nihon Kohden Corporation, Nishiochui Shinjuku-ku, Tokyo, Japan; and 4Department of Physiology, Louisiana State University Health Sciences Center, New Orleans, Louisiana

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Toyoa, Eiji, Yasuo Ogasawara, Osamu Hiramatsu, Hiroyuki Tachibana, Fumihiko Kajiya, Shinji Yamamori, and William M. Chilian. Dynamics of flow velocities in endocardial and epicardial coronary arterioles. Am J Physiol Heart Circ Physiol 288: H1598–H1603, 2005. First published November 18, 2004; doi:10.1152/ajpheart.01103.2003.—The subendocardium is the most vulnerable area of the left ventricle to the effects of hypoperfusion and ischemia. Despite this well-acknowledged observation, the mechanisms underlying this susceptibility are not elucidated, although numerous explanations including differences in transmural distribution of hemodynamics, metabolism, and wall stresses have been proposed. Our goal was to make dynamic measurements of endocardial and epicardial flow velocities, which reflect hemodynamic and wall stresses, to approach this problem. We measured blood flow velocities in subendocardial and subepicardial coronary arterioles of in vivo beating canine hearts using a high-speed, charge-coupled device, intravital videomicroscope with a rod-probe lens. Subendocardial flow was characterized by remarkable systolic flow-velocity reversal (systolic videomicroscope with a rod-probe lens). Subendocardial arterioles during systole have not been used to explain the subendocardial vulnerability to ischemia, because direct observation of retrograde flow in subendocardium has not been evident.

We reason that the amount of subendocardial blood retrograde flow may be an important consideration, because in some respects, it is a wasteful flow, a so-called “slosh,” that reflects the net perfusion as well as the time for perfusion. Accordingly, we also believe that the retrograde systolic blood flow contributes to the vulnerability of the subendocardium to ischemia.

METHODS

Animal preparation. Experimental procedures and protocol were conducted according to the institutional guidelines approved by the Animal Research Committee of Kawasaki Medical School (nos. 95013, 96003, and 97058). Adult mongrel dogs (n = 25) of either sex that weighed 18–32 kg were fully anesthetized with pentobarbital sodium (30 mg/kg iv) and ventilated by a respirator pump (model VS600; Instrumental Development). ECGs were recorded from standard limb leads, and a pressure catheter (model SP674; Millar) was inserted via the right carotid artery to measure the aortic and left ventricular (LV) pressures. After median sternotomy and left thoracotomy were performed, the heart was exposed, supported in a pericardial cradle, and paced at 120 beats/min via atrial pacing. A diagonal branch of the left anterior descending artery (LAD) was catheterized for intracoronary injection of the microflow tracers. Coronary blood flow (CBF) of the LAD was measured using a transit-time ultrasonic flowmeter (model T206; Transonic Systems). Systemic hemodynamics and CBF were recorded on a data recorder (LabView 3.1.1; National Instruments Japan; Tokyo). As the experiment was completed, each dog was killed by induction of fibrillation after additional administration of pentobarbital sodium.

High-speed, charge-coupled device, intravital videomicroscope. To make measurements of microvascular flow velocities (frame-by-frame movement of the tracers), we used a high-speed, charge-coupled device (CCD), intravital videomicroscope system (model MU925S-11; Nihon Kohden; Tokyo; Fig. 1). In this camera, the CCD image sensor has 130,000 pixels, a visual field of 1.04 × 0.80 mm².
the heart. Measurements of blood flow velocity and diameter. The rod probe of the CCD camera was introduced onto the LV endocardial surface at the midportion of the intraventricular septum via the mitral valve to visualize subendocardial coronary arterioles. In separate experiments, the probe was positioned on the heart surface to make measurements in the subepicardial arterioles. As indicated by the focus range of the camera, arterioles were located at depths between 0 and 250 μm from both endo- and epicardial surfaces. The sizes of the arterioles were 35–160 μm in subendocardium and 50–155 μm in subepicardium. After identification of a suitable field (straight segments) within visible arterioles, microflow tracers were administrated (0.8-ml intra-coronary bolus) and followed by a saline flush (2 ml). This tracer administration occurred only once per experiment. Flow-velocity measurements were restricted to the first several cardiac cycles after administration of the tracer bolus. Microflow tracers (lot 5010; Sekisui; Aichi, Japan; Ref. 20) had the following characteristics: diameter, 4–8 μm; specific gravity, 1.36; consisting of fine (0.2 μm) particles of zirconium that reflect halogen light to facilitate visualization. The microflow tracers (4–8 × 10^6 particles/ml) were suspended in 0.09% sodium lauryl sulfate (Nacalai Tesque; Kyoto).

Among the flow tracers appearing in the vessels, tracers of the highest speeds (likely those in the center of the flow stream) were used to calculate flow velocity. Each tracer in the target arteriole was tagged, and its coordinate (center of tracer) was measured frame by frame (Fig. 2). Flow velocity was then calculated after correction for tissue movement from reference points; i.e., vascular bifurcation and/or solitary, embolized tracers. Phases of the cardiac cycle were precisely identified by referring to the ECG and aortic pressure waveforms on the high-speed image monitor in real time, and each calculated velocity value was placed in the context of the cardiac cycle. Velocities slower than 50 mm/s were considered as reliable, because tracer images faster than that were subject to some image distortion. Finally, the intervals of the fastest velocities at each phase were interpolated by spline function. The flow-velocity waveform during systole was divided into two components, namely, the systolic reverse-flow velocity component (SR) and the systolic forward-flow velocity component (SF). Values for the systolic slosh ratio, which was calculated as SR area/(SF area + SR area), were compared between subendocardial and subepicardial sites.

Phasic diameter measurements of arterioles were obtained offline, frame by frame, using an automatic analysis program (NIH Image 1.61) as we have previously described (9, 29, 30). Pulsation amplitude values (in percent) of the arterioles in the cardiac cycle were analyzed as [(diastolic diameter – systolic diameter)/diastolic diameter] × 100. Measurements of velocity (slower than 50 mm/s) and diameter were performed independently by two experts. Measurement accuracy was established by ascertaining the interobserver differences for several randomly selected frames. The correlation coefficients for the velocity and diameter values were 0.92 and 0.95, respectively.

**Statistical analysis.** Data are expressed as means ± SE. Comparisons of systolic slosh ratios and pulsation amplitudes between subendocardial and subepicardial arterioles were performed by unpaired t-test. Statistical significance was set at P < 0.05.

**RESULTS**

All hemodynamic variables and CBF values obtained during the measurements were as follows: aortic pressures, 110.1 ± 14.8/84.8 ± 20.6 mmHg; systolic LV pressure, 124.9 ± 8.5 mmHg; LV end-diastolic pressure, 12.2 ± 8.9 mmHg; heart rate, 118.3 ± 6.1 beats/min; and mean CBF, 33.8 ± 16.0 ml/min.

Figure 2 shows images of subendocardial coronary arterioles with flow-velocity tracers. Six samples of subendocardial flow velocity and diameter change were calculated. An example of tracings from subendocardial arterioles (Fig. 3, top) revealed inflow predominance during diastole and reverse flow during...
systole. In the other five cases, diastolic velocity exceeded 50 mm/s and could not be measured using our technique. Therefore, we focused on velocity patterns during systole (Fig. 4, top). Often we observed two components of reverse flow: one during early systole and a second during late systole. Six samples of subepicardial coronary flow revealed a diastolic predominance, and furthermore, a larger component of systolic forward flow (Figs. 3 and 4, bottom).

In subendocardial arterioles, a significant amount of flow was retrograde during systole; systolic slosh ratio was 84.2 ± 8.0% (Table 1). This contrasts with the subepicardial arterioles (ratio, 24.6 ± 7.9%), which was significantly smaller than in the subendocardium (P < 0.0005). In both sites, maximum

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Fig. 2. Images of the subendocardial coronary arterioles with flowing micro-flow tracers in the beating canine heart. Time sequences of the images are displayed from top to bottom (5 ms/frame). Same tracer (arrow) shows deceleration of forward flow at the beginning of systole (top four photos), then flow cessation, and then a change to backward flow (bottom three photos). Bar indicates 250 μm (analyzed maximal distance for tracer movement per frame). R, R wave on ECG.

Fig. 3. An example of tracings of the flow-velocity waveforms and vascular diameter changes of the subendocardial (top) and subepicardial (bottom) arterioles. These samples were rare but enabled us to observe maximum forward- and reverse-flow velocities throughout the cardiac cycle. Note the difference in pattern of the waveforms. During systole, subendocardial flow was characterized by retrograde flow with less forward flow and was accompanied by a decrease in vascular diameter. Subepicardial flow was characterized by predominant systolic forward flow, a small component of reverse flow, and a small change in diameter.

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Fig. 4. Images of the subendocardial coronary arterioles with flowing micro-flow tracers in the beating canine heart. Time sequences of the images are displayed from top to bottom (5 ms/frame). Same tracer (arrow) shows deceleration of forward flow at the beginning of systole (top four photos), then flow cessation, and then a change to backward flow (bottom three photos). Bar indicates 250 μm (analyzed maximal distance for tracer movement per frame). R, R wave on ECG.
Diastolic flow velocities were not measurable because they were off the scale. Measurable velocity of systolic reverse flow in subendocardium ($-41.1 \pm 5.8$ mm/s) was higher than in subepicardium ($-19.8 \pm 6.6$ mm/s; $P < 0.05$). The pulsation amplitudes of diameter in subendocardial and subepicardial arterioles were $24.5 \pm 8.7$ and $2.6 \pm 4.4\%$, respectively ($P < 0.05$); this is consistent with our previous report (29).

Table 1. Comparison of systolic reverse flow and decrease in diameter between subendocardial and subepicardial arterioles

<table>
<thead>
<tr>
<th>Subendocardial Arterioles</th>
<th>Subepicardial Arterioles</th>
<th>$P$ Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic slosh ratio, %</td>
<td>$84.2 \pm 8.0$</td>
<td>$24.6 \pm 7.9$</td>
</tr>
<tr>
<td>Pulsation amplitude, %</td>
<td>$24.5 \pm 8.7$</td>
<td>$2.6 \pm 4.4$</td>
</tr>
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Values are means $\pm$ SE. Systolic slosh ratio ($\%$) = $100 \times$ (systolic reverse-flow component)/(systolic reverse-flow component + systolic forward-flow component). This ratio signifies the component of flow that is retrograde, which must be refilled during subsequent diastole. Flow velocity less than $\pm 50$ mm/s was exclusively measured because of temporal resolution; note that flow component in diastole ($>50$ mm/s in velocity) was not included in the formula. Pulsation amplitude ($\%$) = $100 \times$ (diastolic diameter − systolic diameter)/(diastolic diameter).

**DISCUSSION**

In the present study, we directly compared subepicardial and subendocardial coronary arteriolar dynamics during the cardiac cycle to better understand the possible mechanisms that underscore vulnerability of the subendocardium to coronary hypoperfusion. We observed a substantial component of systolic retrograde flow velocity in the subendocardial arterioles that was much larger than in the subepicardium. This phenomenon has been hypothesized in many previous studies (4–7, 10, 12, 18, 25), but the present study confirmed this hypothesis as the first systemic evaluation. The volume of retrograde flow represents an amount that must be refilled during the subsequent diastolic period and thus detracts from overall antegrade perfusion in the subendocardium. This effect of contraction on subendocardial flow may contribute to the vulnerability of this region to coronary hypoperfusion (11). In this manner, as coronary reserve is diminished, the ability of the subendocardial vasculature to dilate as compensation for the loss of flow is hampered because of the retrograde pumping (2). Thus we believe our data provide important insight into the cause of subendocardial vulnerability to ischemia.

Mechanisms of retrograde flow from endocardium to epicardium can be explained by transmural differences in cardiac

![Fig. 4. Examples of systolic flow-velocity waveforms and diameter changes of the subendocardial and subepicardial coronary arterioles. These were focused on systolic phase, because diastolic velocity was off the scale. Systole is indicated by the period between two dashed lines within the graphs. Massive retrograde flow was observed during systole in subendocardial arterioles, whereas a smaller reverse-flow component was observed in subepicardial arterioles.](http://ajpheart.physiology.org/)
contraction. In early systole (during LV pressure development), the difference in the systolic flow pattern between both layers is well explained by the intramyocardial pump model (25). Direct measurements of intramyocardial pressure in the beating canine heart showed that the systolic intramyocardial pressure-to-LV pressure ratio decreased linearly from endocardium to epicardium (8). Accordingly, the intramyocardial blood inflow volume that occurred during diastole was squeezed out retrogradely.

Reverse flow in the later phase of systole has been explained by the time-varying elastance model (18). The elastance model predicts that retrograde pumping occurs because of time-varying elastance during the cardiac cycle, which is highest at the end of systole. Our results further refine these ideas. Specifically, we predict that because of the lower perfusion pressures in the subendocardium (2), the effects of elastance would be greatest in this region and would lead to higher retrograde systolic flow than in the subepicardium. We speculate that enhancement of late-systolic retrograde flow would be observed under pronounced hyperkinetic status and also during hypoperfusion.

Another factor that possibly influenced our results is that the arterioles in the endocardium are located at the most inner layer of the myocardial wall. Although these vessels are not embedded in the myocardium, they are situated on the endocardial surface and are likely affected by LV pressure. Their phasic diameters would also reflect influences imposed on the feeder and drainage vessels that are embedded in the intramyocardial musculature in close proximity to the endocardial surface. Another factor that may produce retrograde flow in the arterioles at the subendocardial surface is myocardial strain. Kimura et al. (16) demonstrated drastic systolic flow deceleration in the atrium, where intramyocardial pressure linked to atrial pressures is low, so that myocardial deformation is a candidate. Partition of the influences of LV pressure, intramyocardial pressure, and myocardial strain in the mechanism(s) of this flow reversal is not yet elucidated; however, endocardial vessels likely represent a suitable model for studying the interactions of contraction with microvascular hemodynamics.

Another interesting finding of the present study was observed during the presystolic phase. Flow-velocity deceleration started immediately before the R wave on the standard limb-lead ECG, and this deceleration led to flow reversal in early systole. Ashikawa et al. (1) also reported the same abrupt decline and momentary reverse flow in epicardial small arterioles during the prejection period. The mechanism of the deceleration at this phase is unclear, although an increase in end-diastolic LV pressure by atrial kick, LV stretch by atrial kick, and LV myocardial deformation are candidates. Taken together, cardiac phase-dependent mechanisms and the partitioning of 1) LV pressure, 2) intramyocardial pressure, 3) elastance, and 4) myocardial deformation resulting in systolic flow reversal are still not elucidated.

In the subepicardium, we observed a fraction of systolic retrograde flow in most of the preparations (Fig. 4, bottom). This component is normally concealed by the capacitance of the extramural superficial coronary arteries (26).

Limitations of the study. With the specifics of our system and the characteristics of the rod-probe microscope, we have found that 15-μm-diameter, zirconium microflow tracers are ideal for our application, because they are very reflective and thus are easily visualized. However, we would like to mention the possibility that the flow tracer could affect arteriolar flow dynamics by embolization, because the tracer is larger in diameter than the capillaries. To minimize this potential complication, tracers were administered once per experiment and were not repeated. Flow-velocity values during the first few seconds (when the tracers appeared) were equivalent to those measured toward the end of the sequence. If embolization was severe, there should have been a progressive reduction in velocity during the sequence; but this was not observed. Smaller tracers (diameter, 4 μm) of the same material flowing in the blood vessels were too dim to visualize. Furthermore, our preliminary studies taught us that 0.8 ml of a flow tracer of 4–8 × 10^9 particles/ml (thus 3.2–6.4 × 10^6 as the total number of particles) was the minimum number to be used to obtain a reliable velocity waveform line by spline plotting. Nevertheless, we believe it is fair to compare flow waveforms between subendocardial and subepicardial arterioles that are evaluated by an identical dose of flow tracer.

A doughnut-shaped balloon on the probe tip was used to keep a space between the cardiac surface and the probe lens, and it functioned as a cushion to avoid compression around the observed vessels. This became more effective by smooth-probe manipulation by well-trained experts, who could synchronize probe movements with heart beats. The construction and procedures of the present system were basically the same as those we used with our previously developed intravital videomicroscope (9, 29, 30) but newly loaded with a high-speed CCD camera. The original article (29) describing use of the previous system demonstrated that less difference in pressure waveform value was observed between the inner space of the balloon and the outer space (intra-LV cavity) during observation of the subendocardial vessels, which indicated that compression on observed vessels was negligible. Although we may not perfectly exclude any compressions, we believe it is fair to compare velocity and diameter values between subepicardium and subendocardium that are observed by identical manipulation.

Our microscope has a 6.5-mm-diameter rod-probe lens. This probe was inserted from the left appendage and through the mitral valve of an in vivo beating heart to visualize subendocardial flow. Probe insertion sometimes affected systemic hemodynamics depending on the heart size and the location of target arterioles. Nevertheless, under these conditions, arterioles in the subendocardium showed more enhanced systolic retrograde flow compared with the subepicardium, where flow was observed without any alterations in LV contractility.

Although the slosh ratio was calculated as the area under the curve of measurable velocity, we may have underestimated the magnitude because of technical limitations in our system. We could not measure velocities greater than 50 mm/s; thus our measurements have some error. Nevertheless, we believe our conclusion that enhanced slosh occurs in subendocardial vs. subepicardial arterioles.

In conclusion, we succeeded in visualizing the subendocardial coronary arteriolar dynamics and measuring diameter and flow velocity of in vivo beating canine hearts using our newly developed rod-probe, high-speed CCD, intravital videomicroscope. Prominent retrograde flow, the “coronary slosh phenomenon” in the subendocardium, may be the basis of subendocardial vulnerability to ischemia and hypoperfusion.
ENDOCARDIAL AND EPICARDIAL CORONARY FLOW DYNAMICS

GRANTS

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REFERENCES


