A novel strategy for increasing wall thickness of coronary venules prior to retroperfusion

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Submitted 7 March 2006; accepted in final form 22 March 2006

Choy, Jenny Susana, and Ghassan S. Kassab. A novel strategy for increasing wall thickness of coronary venules prior to retroperfusion. Am J Physiol Heart Circ Physiol 291: H972–H978, 2006.—The sudden exposure of veins to arterial pressures during coronary venous retroperfusion may cause rupture of small venules. Our rationale is to first occlude the coronary vein, which will cause an increase in pressure intermediate to arterial and venous values, and hence lead to remodeling and increased wall thickness of the veins prior to retroperfusion. To accomplish this objective, five pigs were subjected to left anterior descending (LAD) vein ligation while six pigs served as sham. Myocardial tissue samples were obtained from the area adjacent to the LAD vein at four transmural locations of the left ventricular free wall: epicardial surface, subepicardium, midmyocardium, and endocardium. Arterioles and venules from the experimental and sham control groups were photographed, and the following measurements were made: inner and outer circumferences, inner and outer areas, major and minor diameters, and intima-media thickness. Each vessel was categorized in four different orders according to lumen diameter. Our results show that intima-media thickness was larger in the experimental group in all four regions of the heart and in all four orders of the vessels, although venules from the epicardial region showed the largest increase in thickness. The intima-media thickness-to-radius ratio was also larger in the experimental group and decreased from epicardial to endocardial region of the heart and from order 1 to order 4 of the vessels. The present study provides a rationale for the development of coronary retroperfusion strategy that avoids vessel rupture and hemorrhage in the postcapillary venules.

METHODS

Tissue specimens were obtained from 11 swine hearts recently used in the study of large veins (9). Briefly, five pigs, 3 to 4 mo old, underwent ligation of the great cardiac vein close to the LAD vein, and six served as sham controls. After 2 wk of ligation, the animals were euthanized, and the coronary vessels were perfusion fixed with 6.25% glutaraldehyde solution at 1,100 mOsm and a pressure of 100 mmHg. The LAD vein was cut perpendicular to the long axis of the vessel, and detailed morphometric measurements were made along the vein from the point of ligation near the base down to the apex of the heart as recently reported (9). The hearts used in the present study were fixed and stored in the refrigerator in 6.25% glutaraldehyde solution.

Histological preparation of tissue specimens. Myocardial tissue samples were obtained from the area adjacent to the LAD vein at four transmural locations of the left ventricular free wall: epicardial surface, subepicardium, midmyocardium, and endocardium. The latter three regions were defined by dividing the wall thickness into three equal portions (subepicardium, midmyocardium, and endocardium). Each region was sliced under the microscope, using ordinary razor blades, into ~2 × 2 × 2 mm uniform pieces along the long axis of the

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myocardial fiber. The samples were then rinsed three times with a buffer solution and processed by dehydration in increasing concentrations of alcohol (70, 80, 95, and 100%). The samples were then embedded in glycol methacrylate (JB-4 solution) in such a way that transverse cross-sectional slices could be made. Tissue blocks were carefully oriented in the specimen holder of the microtome with the long axes of the myocardial fibers perpendicular to the glass knife. Sections of 3 μm thickness were cut by using a conventional microtome (HM 340 E from Microm), mounted on glass slides, and stained with 0.1% Toluidine blue for assessment of arteriolar and venular morphometry by light microscopy.

Morphometric measurements. Each section was completely surveyed for arterioles and venules. Measurements were restricted to those vessels that satisfied the following criterion: no tears or sectioning artifact, an intact layer of the tunica media, and a nearly circular profile (difference between minor and major axes <10%) for arterioles of orders 1–4 and for venules of orders 1 and 2. The circularity condition was relaxed for venules of orders 3 and 4 (<25%), provided that the wall thickness was uniform circumferentially. Arterioles and venules were distinguished at the time of measurement. Arterioles have a more defined muscular tunica media, an obvious intima delineated by endothelial cells, and stain lighter with Toluidine blue as compared with venules. Photographs of histological sections of acceptable arterioles and venules were taken with a Spot Insight Color Digital Camera (Diagnostic Instruments) attached to the microscope (Nikon Eclipse E600). For each vessel (either venule or arteriole), the following measurements were made with the use of SigmaScan Pro 5 software: inner and outer circumferences, inner and outer areas, major and minor diameters, and intima-media thickness (IMT) at four different locations around the vessels (north, south, east, and west). The average of the IMT was used over the four measurements. A circular diameter of the vessels was calculated from the inner circumference divided by 3.14. Each vessel was categorized in four different orders according to a diameter range previously determined by Kassab et al. (22): 10–13.5 (order 1), 13.6–23.1 (order 2), 23.2–38.1 (order 3), and 38.2–80.4 μm (order 4). All measurements were made from photographs taken at ×1,000 magnification (spatial resolution of 0.07 μm), with all values expressed in micrometers.

Statistical analysis. All morphometric data were reported as means ± SD. Comparisons of data between experimental and sham control groups for both arterioles and venules were made with Student’s t-test. A value of P ≤ 0.05 was considered significant.

RESULTS

A total of 1,903 vessels were analyzed: 696 venules and 282 arterioles corresponded to the sham control group, and 693 venules and 232 arterioles corresponded to the experimental group. Arterioles from both sham and experimental groups were not significantly different and were combined together.

Figure 1 shows cross sections of venules from the epicardial and subepicardial regions of the heart in the sham control (left) and experimental (right) groups, respectively. The larger IMT in the venules from the experimental group can be noted when compared with the venules from the sham control group of similar diameter or order.

The IMT of arterioles from both groups combined together and of venules from the experimental and sham groups of various orders in the four regions of the myocardium are shown in Fig. 2. The numbers in parentheses reflect the percent increase of the IMT in the venules from the experimental as compared with the sham control group. The values for IMT were significantly larger in the experimental group compared with sham control group in all four regions of the myocardium (P < 0.001). Experimental venules of order 4 from the epicardial surface showed the largest change in IMT (166%) when compared with venules from the sham control group. Change of IMT decreases from epicardium to endocardium and in-

**Fig. 1.** A: cross section of a venule of order 4 from epicardial surface of the heart in sham control group (Toluidine blue at ×600). B: cross section of a venule of order 4 from epicardial surface of the heart in experimental group (Toluidine blue at ×600). C: photomicrograph of a venule of order 3 from subepicardial region of the heart in sham control group (Toluidine blue at ×1,000). D: photomicrograph of a venule of order 3 from subepicardial region of heart in experimental group (Toluidine blue at ×1,000). V, venule.
creases from order 1 to order 4. We found no differences in the IMT of capillaries (<10 μm) from the experimental and sham control groups (data not presented).

Figure 3 shows the IMT-to-radius (IMT-to-R) ratio of arterioles from both groups and of venules from the experimental and sham control groups of various orders in the four regions of the myocardium. The values for the IMT-to-R ratio decrease from epicardium to endocardium and from order 1 to order 4. The values were significantly larger in the experimental group than in the sham control group (P < 0.001).

Frequency histograms of the IMT and the IMT-to-R ratio in each region of the left ventricular wall for combined arterioles and venules for both experimental and sham groups are shown in Figs. 4 and 5, respectively. In all four regions of the myocardium, there is a right shift in the distribution of the vessels from the experimental group. The statistics are summarized in Tables 1 and 2 for IMT and IMT-to-R ratio, respectively. The differences in the mean values between the experimental and sham groups for the combined vessels are statistically significant in all four regions of the heart for the IMT (Table 1) and in the epicardial, subepicardial, and midmyocardial regions for the IMT-to-R ratio (Table 2). The coefficient of variance CV (CV = SD/Mean) was calculated for both distributions. It is apparent that CV decreases for the
experimental group in all four regions of the heart, suggesting arterialization of the microvasculature.

**DISCUSSION**

*A novel strategy for prearterializations of coronary veins for retroperfusion.* Because coronary veins do not typically show arteriosclerotic changes (8, 23, 24), which is likely due to the lower pressure, revascularization of these vessels to nourish a particular region of the myocardium is a promising approach. The creation of conduits for revascularization must take into consideration several key factors, such as the adequacy of blood supply to the region of interest, the changes in the hemodynamic parameters (pressure and flow) of supply and receive vessels, the homeostatic mechanical status, and the subsequent remodeling process in the respective vessels.

In a recent study (9), we focused on the mechanical adaptation of the large coronary veins in response to an increase in pressure due to ligation. We found a significant increase in wall thickness of large veins to restore the intramural circumferential stress. Furthermore, we found that large veins develop intimal thickening only at points where myocardial support is lacking and circumferential stress and strain are higher than...
regions that have myocardial support. Hammond et al. (18) have shown that severe damage to venules begins at a pressure >60 mmHg. Hence the adaptation of the coronary veins to intermediate pressures before being exposed to arterial pressures is very important in venous retroperfusion. The present study demonstrates that the proposed ligation arterializes microvessels to potentially prevent the rupture of those vessels during retroperfusion. The greater arterialization of epicardial venules is fortuitous because those vessels experience larger transmural pressures during the cardiac cycle due to the lower intramyocardial pressure as compared with endocardial vessels (19).

A two-stage procedure similar to that suggested in our study was first attempted by Beck and colleagues (3–5). Their first stage consisted of the placement of a free vein graft between the coronary sinus and the aorta, and the second stage of the operation (3 wk after the first operation) consisted of partial ligation of the coronary sinus at the ostium of the sinus into the right auricle. This was done to prevent overpressurization of the coronary sinus system. Their goal was to allow the venous system some time to arterialize before exposing it to the systemic arterial pressure. Although Beck and colleagues (3–5) had some success with this procedure, the efficacy of the procedure was limited, in part, because of the site of arterialization. Beck et al. (3–5) arterialized the coronary sinus, which has numerous interconnections with the Thebesian vessels that drain blood directly into the chambers of the heart. Hence the majority of arterial blood flow was shunted into the chambers of the heart via these interconnections. It is now well established that the number of interconnections with the Thebesian vessels is far less in the LAD vein than in the coronary sinus (3), which will reduce the shunting of flow into the chambers and deliver more of the arterial blood to the venules and subsequently the capillaries. Hence in our studies, we pursue segmental or selective venous arterialization rather than the global arterialization proposed by Beck.

Remodeling of coronary venules. The microvasculature is a highly adaptable network capable of structural and functional changes in response to changes in flow and pressure. Mechanical stresses like shear and circumferential wall stress dictate microvascular remodeling (32). Venules can increase their wall thicknesses in response to chronic pressure elevation (25) and can change their wall structure, length, and diameter after chronic stimulation (1). It is well established that hypertension induces an increase in the arterial wall thickness as a result of medial hypertrophy and hyperplasia and the deposition of extracellular matrix (20). Similarly, when veins are exposed to arterial pressures, the result is an increase in medial thickness attributed to the pressure-induced elevation of circumferential tension and stress (10, 16, 17, 31). The increase in pressure may also lead to an increase in flow and wall shear stress that results in vasodilatation and remodeling of the vessel wall (28). The increase in pressure may make veins prone to atherosclerotic changes due to migration and proliferation of smooth muscle cells into the intima, especially in large veins (29).

Increases in flow during venous retroperfusion, however, would likely reduce the intimal hyperplasia and atherogenesis.

The transmural differences in coronary blood flow and pressure may contribute to the differential remodeling in various regions of the myocardium as observed in the present study. Chilian (7) has reported that pressure and resistance in arterioles, capillaries, and venules are different across the wall of the left ventricle. The reasons for these transmural differences are likely due to distribution of mechanical stresses and myocardial oxygen demands. Our results suggest that pressure in the coronary venous system, after ligation, is different in the four regions of the heart. It is expected that the venous pressure at the epicardial surface would be highest for two reasons: 1) lower extravascular myocardial stress and 2) higher intravascular pressure, because those vessels are farthest from the shunting subendocardial region. This would explain the different degree of remodeling observed across the left ventricular wall and in different size vessels. The second reason may be augmented by the presence of venoluminal vessels that drain blood from the epicardial veins directly into the venticles (13).

Significance of the architecture of venous vessels for retroperfusion. The venous drainage of the heart involves two systems, the greater and the smaller systems, with a network of venovenous communications between these two. The greater system includes vessels that drain into the coronary sinus. The smaller system comprises the Thebesian vessels that drain directly into the cardiac chambers. They communicate with the coronary veins and arteries through the capillary network and can drain a significant amount of venous efflux (2, 27, 34). The Thebesian vessels are capable of draining up to 50% of coronary blood flow in a beating heart (33). The significance of this system may be realized in its ability to provide alternative routes for venous drainage if portions of the venous system are being used for other purposes such as percutaneous in situ venous interventions (11). If the LAD vein or the great cardiac vein is obstructed, then the numerous anastomosis and side channels between the venous systems ensure that venous drainage is not interrupted. As one ligates the coronary venous system closer to the sinus and additional ligations are made to other epicardial veins, more significant perturbation occurs in the flow and function of the heart. Although the Thebesian venous system may be capable of off-loading some of this flow during obstruction of the superficial veins, experiments show that the diffuse nature of the epicardial venous system itself

<table>
<thead>
<tr>
<th>Region</th>
<th>Experimental IMT, μm</th>
<th>Sham IMT, μm</th>
<th>%CV</th>
<th>%CV</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epicardium</td>
<td>1.44±0.72</td>
<td>0.90±0.81</td>
<td>49.8</td>
<td>89.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Subepicardium</td>
<td>0.95±0.58</td>
<td>0.62±0.62</td>
<td>60.7</td>
<td>76.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Midmyocardium</td>
<td>0.76±0.45</td>
<td>0.63±0.51</td>
<td>59.1</td>
<td>81.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Endocardium</td>
<td>0.71±0.47</td>
<td>0.62±0.44</td>
<td>65.9</td>
<td>71.4</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Values are means ± SD. IMT, intima-media thickness. Coefficient of variance (%CV) is determined as %CV = SD/mean × 100.

Table 2. IMT-to-radius ratio of combined arterioles and venules in different regions of myocardium

<table>
<thead>
<tr>
<th>Region</th>
<th>Experimental %CV</th>
<th>Sham %CV</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epicardium</td>
<td>0.043</td>
<td>0.057</td>
<td>37.0</td>
</tr>
<tr>
<td>Subepicardium</td>
<td>0.059</td>
<td>0.066</td>
<td>66.1</td>
</tr>
<tr>
<td>Midmyocardium</td>
<td>0.066</td>
<td>0.082</td>
<td>59.6</td>
</tr>
<tr>
<td>Endocardium</td>
<td>0.053</td>
<td>0.058</td>
<td>55.1</td>
</tr>
</tbody>
</table>

Values are means ± SD. CV is determined as %CV = SD/mean × 100.
also provides an important system for alternative drainage (14). The numerous interconnections or anastomosis between the Thebesian and epicardial venous systems ensure an alternative path for venous return in the presence of ligation.

Critique of methods. Several limitations of the present study warrant discussion. Although we were unable to measure the distribution of intravascular pressure across the left ventricular wall, it is reasonable to expect a decrease in pressure from epicardium to endocardium in line with the degree of venting of the LAD vein through the interconnections between the epicardial veins and the Thebesian vessels. The ligation of the LAD vein raises the pressure to ~50 mmHg in the territory close to the occlusion, but it must decrease in other regions of the myocardium due to the presence of venulomalous vessels that drain blood from the superficial veins directly into the cardiac chambers. This anatomical observation would explain the differences in the magnitude of the remodeling process of the epicardial, subepicardial, midmyocardial, and endocardial venules as well as in different orders of vessels.

Histological differences between large arterioles and venules (>100 μm in diameter) are well established in the literature under normal conditions (21). Remodeled venules, however, may be erroneously identified as arterioles because of the increase in their wall thickness. On the basis of some histological differences and color staining with Toluidine blue, we were able to distinguish arterioles from venules. In case our distinction of vessels was erroneous, we constructed frequency histograms of combined arterioles and venules to evaluate the increase in wall thickness and the homogenization of blood vessels in the experimental group. The latter approach did not require distinction of arterioles and venules but still demonstrates a right shift of probability density of the experimental group as well as a decrease in the variance of the distribution. Both features suggest arteriolarization of venules and structural homogenization of the microvasculature.

Although we did not perform immunohistochemical analysis to determine the changes in smooth muscle mass as compared with collagen and extracellular matrix, our previous observations (9) on large coronary veins suggest that the increase in medial thickness may involve both factors. Intengan and Schiffrin (20) have documented the remodeling of structural and mechanical properties of arteries in response to hypertension. More extensive studies on venous remodeling, however, are needed.

Although we did not measure cardiac function in the present group of experimental animals, we did not observe any hemodynamic or electrophysiological changes in the hearts in response to a single ligation of the LAD vein. Furthermore, we did not find any histological evidence of myocardial injury or inflammatory process in the microcirculation in response to ligation of the LAD vein. Previously, Gross et al. (15) have shown that prior venous ligation reduces the mortality rate from subsequent coronary ligation.

In conclusion, the potential of the coronary veins to act as conduits for revascularization has been evaluated by different investigators for more than a century. The major hurdle has been the damage of venules during sudden exposure to arterial pressure. The present study initiates a program that will serve to eliminate the edema and hemorrhage that result during venous retroperfusion as the pressure is suddenly increased to arterial values. The rationale is to increase the venous pressure to arterial values in two steps: 20 to 50 mmHg for 2 wk to allow arterialization of the veins, followed by 50 to 100 mmHg. The significance of the present study is that the occlusion of the LAD vein arterIALIZES the small venules of the left ventricle, which is important to prevent the rupture of these vessels during retroperfusion. The present findings are essential for a rationale design of coronary venous retroperfusion.

REFERENCES


