Nonuniformity of axial and circumferential remodeling of large coronary veins in response to ligation

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Submitted 31 August 2005; accepted in final form 16 November 2005

Choy, Jenny Susana, Quang Dang, Sabee Molloi, and Ghassan S. Kassab. Nonuniformity of axial and circumferential remodeling of large coronary veins in response to ligation. Am J Physiol Heart Circ Physiol 290: H1558–H1565, 2006. First published November 18, 2005; doi:10.1152/ajpheart.00928.2005.—The pressure-induced remodeling of coronary veins is important in coronary venous retroperfusion. Our hypothesis is that the response of the large coronary veins to pressure overload will depend on the degree of myocardial support. Eleven normal Yorkshire swine from either sex, weighing 31–39 kg, were studied. Five pigs underwent ligation of the left anterior descending (LAD) vein, and six served as sham-operated controls. The ligation of the coronary vein caused an increase in pressure intermediate to arterial and venous values. After 2 wk of ligation, the animals were euthanized and the coronary vessels were perfusion-fixed with glutaraldehyde. The LAD vein was sectioned, and detailed morphometric measurements were made along its length from the point of ligation near the base down to the apex of the heart. The structural remodeling of the vein was circumferentially nonuniform because the vein is partially embedded in the myocardium; it was also axially nonuniform because it is tethered to the myocardium to different degrees along its axial length. The wall area was significantly larger in the experimental group in proportion to the increase in pressure. The major conclusion of this study is that the remodeling of the veins in response to pressure overload, however, are much less common. Indeed, we are unaware of any studies on the remodeling of the coronary veins in response to pressure overload. This is unfortunate, however, because pressure-induced remodeling is a central issue in such therapeutic approaches as chronic coronary retroperfusion (20, 21). The objective of the present study is to fill in this gap to understand the mechanical adaptation of the large coronary veins in response to elevated pressures.

Our hypothesis is that an increase in left anterior descending (LAD) vein pressure will lead to remodeling or arterIALIZATION; pressure-overload; retroperfusion; wall stress; surrounding tissue. circumferentially and axially nonuniform because the LAD vein is partially embedded in the myocardium circumferentially and is tethered to the myocardium to various degrees along its axial length. Hence, we will determine the structural remodeling of the LAD vein circumferentially and along its axial length.

The present findings confirm these hypotheses and show significant thickening of the venous wall to restore the intramural circumferential stress. Furthermore, the data show that the LAD vein developed intimal thickening (inward remodeling) only in the absence of myocardial support and that significant medial thickening (hypertrophic remodeling) occurs to restore the intramural circumferential stress. The present study clarifies the structural remodeling in the coronary veins after ligation and provides a rationale for the development of a coronary retroperfusion strategy that avoids long-term atherosclerotic changes.

METHODS

The choice of the present animal model was based on the similarities between human and swine coronary vessels (10). This study was conducted in accordance with national and local ethical guidelines, including the Institute of Laboratory Animal Research Guide; the Public Health Service policy; and the Animal Welfare Act and approved by the Institutional Animal Care and Use Committee of the University of California, Irvine.

Animal preparation. Eleven normal Yorkshire swine of either sex with body weight of 31–39 kg were studied. The animals were fasted overnight; ketamine (20 mg/kg) and atropine (0.05 mg/kg) were administered intramuscularly before endotracheal intubation. The animals were ventilated with the use of a mechanical respirator, and general anesthesia was maintained with 1 to 2% isoflurane and oxygen. The chest was opened through a left lateral, fourth-intercostal-space thoracotomy, and an incision was made in the pericardium with the creation of a sling to support the heart. The LAD vein was located and isolated close to the great cardiac vein, and a suture was placed around the vessel as shown in Fig. 1. An introducer was used to puncture the vein to measure the venous pressure before ligation. With the catheter inside the vessel and fixed to the surface of the heart, the ligature was tightened to measure the increase in pressure after ligation. The catheter was then withdrawn, and the ligature was made permanent to completely occlude the flow through the vein. The chest was then closed in a standard manner, and the animals were allowed to recover. In five animals, the vein was ligated, whereas in six animals, the suture was not tightened and the animals served as sham-operated controls.

The experimental and sham-operated animals were recovered for 15 days. The animals were administered cefazolin (500 mg im) and buprenorphine (0.03 mg/kg) twice a day for the prevention of infection and the management of pain, respectively. At the scheduled time, the animals were anesthetized as described above. The blood pressure was...
measured through a carotid artery, and anticoagulation was induced with heparin (100 IU/kg) through a jugular vein. The animals were then deeply anesthetized followed by an injection of a saturated KCl solution through the jugular vein to arrest the heart. The aorta was clamped to keep air bubbles from entering the coronary arteries, and the heart was excised and placed in a saline solution. The LAD artery, the right coronary artery, and the left circumflex artery were cannulated under saline to avoid air bubbles and perfused with cardioplegic solution to flush out the blood. The heart was then perfused and fixed with 1 liter of 6.25% glutaraldehyde solution at 1,100 mosM and a pressure of 100 mmHg. The fixed hearts were kept in the refrigerator until the day of histological preparation.

Histological preparation of LAD vein. The LAD vein was cut perpendicularly to the long axis of the vessel into 3- to 4-mm serial segments from the point of ligation to the apex of the heart where the vessel had a diameter of ~0.5 mm. A coordinate system was set up such that the fractional longitudinal position (FLP) at the ligature was defined as 0 and that at the distal end (0.5 mm in diameter) was defined as 1, as shown in Fig. 1. The proximal position of every ring was recorded, and each segment was rinsed three times with a buffer solution and processed by dehydration in increasing concentrations of alcohol (70%, 80%, 95%, and 100%). The segments were then embedded in either JB-4 (odd segments) or paraffin (even segments). All samples were processed by using a microtome (HM 340 E from Microm). The JB-4 embedded segments were cut into 3-µm sections, mounted on glass slides, and stained with toluidine blue for morphometric measurements. The paraffin-embedded segments were cut into 5-µm sections, mounted on glass slides, and deparaffinized and stained with Verhoeff’s elastic stain to study the vessel structure. Photographs of the histological cross-sections of each segment were taken with the use of a light microscope (Nikon) and a Spot Insight Color Digital Camera (Diagnostic Instruments).

Morphometric measurements. Photographs of histological cross-sections of the LAD vein were analyzed. The proximal portion of the vessel faced upstream, and the distal portion faced downstream as shown in Fig. 2. For each photograph, the following measurements were made on the proximal portion of the vessel: inner and outer circumferences; inner, outer, and wall areas; and intimal and wall thickness at twelve equally spaced locations (0°, 30°, 60°, . . . 300°,

Fig. 1. Illustration of left anterior descending (LAD) vein showing point of ligation and definition of fractional longitudinal position (FLP).

Fig. 2. Schematic representation of coordinate system used to measure several parameters on a cross-section of the vein.

Fig. 3. A: phasic pressure in LAD vein before and after ligation in one animal. Solid curve represents mean value of phasic trace. B: mean pressure before and after ligation average over each group of animals.
and 330° in a clockwise direction around the circumference of the vessel). The 90° line corresponds to the center of the anterior portion, and the 270° line corresponds to the center of the posterior portion of the vessel in a cross-sectional view as seen in Fig. 2. Hence, the anterior portion of the vessel, surrounded by connective tissue, ranges from 0–180°, whereas the posterior region (surrounded by myocardium) corresponds to 180–360°. Intimal thickness was measured by using the internal elastic lamina as the line of demarcation between the tunica intima and the media adventitia. All measurements were made with the use of SigmaScan Pro 5 software.

**RESULTS**

The phasic and mean pressures in the LAD vein before and after ligation are shown in Fig. 3A from one animal. The mean pressure, averaged over all the animals, in the LAD vein was 20 mmHg before the ligation and 50 mmHg after the ligation ($P < 0.05$), as shown in Fig. 3B.

The length of the LAD vein was 74.0 ± 9.2 mm in the experimental group and 76.9 ± 7.8 mm in the sham-operated control group ($P = 0.59$). All venous tissue samples from the experimental group ranged in diameter from 2.2 ± 0.08 (FLP, ~0) to 0.50 ± 0.14 mm (FLP, ~1). Veins in the sham-operated control group ranged from 3.04 ± 0.3 (FLP, ~0) to 0.64 ± 0.26 mm (FLP, ~1). These differences were statistically significant in the proximal portion of the LAD vein (FLP < 0.20, $P = 0.03$).

Figure 4 shows histological sections of the LAD vein from experimental (Fig. 4, A and C) and sham-operated (Fig. 4, B and D) groups at 90° and 270°. The increase in intimal and wall thickness in the experimental group is obvious. The thicker intima, however, is restricted to the anterior portion of the vessel (0–180°), especially at 90° (Fig. 4A). Figure 5 shows a paraffin section of the intima-thick region of the epicardial LAD vein revealing increased number of cells (many nuclei), as well as an inflammatory process including macrophages and neutrophils.

The mean wall and intimal thickness for the experimental and sham-operated groups are shown in Fig. 6, A and C, and Fig. 6, B and D, respectively. The most anterior portion of the vessel (at 90°, with no surrounding myocardial tissue) showed the largest change in total wall thickness (~300 μm at FLP of ~0 to ~50 μm at FLP of ~1). The most posterior portion of the vessel (at 270°, surrounded by the myocardium), however, showed the smallest change in wall thickness (~125 μm at FLP of ~0 to ~20 μm at FLP of ~1) as shown in Fig. 6, A and B. Intimal thickness was 20 to 25 times larger in the experimental groups at 90° and 270°. The increase in intimal and wall thickness in the experimental group is obvious. The thicker intima, however, is restricted to the anterior portion of the vessel (0–180°), especially at 90° (Fig. 4A). Figure 5 shows a paraffin section of the intima-thick region of the epicardial LAD vein revealing increased number of cells (many nuclei), as well as an inflammatory process including macrophages and neutrophils.

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portion of the vessel showed the smallest change (~30 μm at FLP of ~0 to ~10 μm at FLP of ~1) as shown in Fig. 6, C and D. The cross-section of the vessel in the experimental group was also represented in cross-sectional diagrams at FLP of ~0.025, 0.225, 0.475, 0.725, and 0.975 and in the sham-operated group at FLP of ~0.025 (Fig. 7, top, right).

The values for the wall area were significantly different between the two groups, especially in the proximal 20% of the vessel length (almost double) as seen in Fig. 8. In the experimental group, the values ranged from 1.1 (FLP, ~0) to 0.04 mm² (FLP, ~1), whereas in the sham-operated group, they ranged from 0.7 (FLP, ~0) to 0.02 mm² (FLP, ~1) (P < 0.05).

The luminal area was significantly smaller in the experimental group (P < 0.001) and more evident in the proximal 15% of the vessel length; i.e., 2 to 3 times smaller, as shown in Fig. 9. In the experimental group, the values ranged from 2 (FLP, ~0) to 0.19 mm² (FLP, ~1), whereas in the sham-operated group, they ranged from 7 (FLP, ~0) to 0.3 mm² (FLP, ~1).

The wall thickness-to-radius ratio was 1.5 to almost 5 times larger in the experimental group (P < 0.001); the values ranged from 0.24 (FLP, ~0) to 0.054 (FLP, ~1), whereas in the sham-operated group, they ranged from 0.050 (FLP, ~0) to 0.018 (FLP, ~1), as shown in Fig. 10. The mean circumferential stress was approximated by Laplace’s equation as the quotient of pressure and wall thickness-to-radius ratio. We found the mean values to be 38 ± 26 and 43 ± 18 kPa for the experimental and sham-operated groups, respectively. The differences were not statistically significant (P = 0.72).

The total (lumen plus wall) area was significantly smaller in the experimental group (P < 0.001), particularly in the proximal segments of the vessel length as shown in Fig. 11. The values in the experimental group ranged from 2.9 (FLP, ~0) to 0.2 mm² (FLP, ~1), whereas in the sham-operated group, they ranged from 7.7 (FLP, ~0) to 0.32 mm² (FLP, ~1).

DISCUSSION

The major finding of the present study is that the pressure increase induced by the ligation of the LAD vein leads to remodeling or arterialization of the vein. The structural remodeling is circumferentially and axially nonuniform because the vein is partially embedded in the myocardium circumferentially and is tethered to the myocardium to various degrees along its axial length.
Effect of venous ligation on pressure waveform and flow distribution. If the coronary venous system consisted of only epicardial drainage into the coronary sinus, then the ligation of a vein would result in a stagnation of flow and an increase in venous pressure to arterial levels. On the other hand, if the coronary venous system consisted of only subendocardial drainage into numerous thebesian vessels, then the ligation of a vein would result in an increase in pressure to capillary levels or below. In actuality, the coronary venous system consists of both a major epicardial sinusal venous system and many subendocardial thebesian vessels. Furthermore, there are communications or interconnections within each system and in between the two systems (14). Hence, ligation of a coronary vein is expected to raise the venous pressure to a value intermediate to capillary and arterial pressures, i.e., ~50 mmHg as documented in the present study (Fig. 3B). Further-
more, the pulse pressure is quite large during the cardiac cycle as seen in Fig. 3A. In systole, the peak pressure approaches arterial pressure because the communication between the sinusal and thebesian vessels is interrupted because the thebesian vessels, which are subendocardial, may be compressed by the high extravascular pressure. Hence, the increase in pressure is a measure of the degree of venting of the LAD vein through the interconnections between the sinusal veins and thebesian system. If the ligation were made at a different axial position along the vein, a different pressure increase would be expected. In the present study, we systematically ligated the LAD vein in all hearts (at approximately the same point) where the LAD vein curves into the great cardiac vein as shown in Fig. 1.

The occlusion of a coronary vein not only increases the venous pressure but also causes a redistribution of the flow to the numerous communications between the various segments of the coronary circulation. Scharf et al. (22) found that the occlusion of the coronary sinus caused a redistribution of the venous flow to other channels emptying in the right heart. Furthermore, they found that the distribution of venous outflow changed when the pressure exceeded 9 mmHg. Hence, we expect significant redistribution of blood flow in the present experimental model.

**Effect of ligation and pressure overload on coronary veins.**

The vasculature is an adaptable structure that is capable of biochemical, architectural, and functional adjustments in response to changes in mechanical stimuli. An increase in blood pressure leads to an increase in tensile stress, which is a stimulus for vascular growth and remodeling (12). In in vitro studies (6), it has been shown that venous smooth muscle cells are much more responsive to mechanical stimuli than their arterial counterparts. Furthermore, if the increase in circumferential stress is too large and abrupt, it can also elicit an injury response. The injury response includes neointimal hyperplasia as a result of the proliferation and migration of the smooth muscle cells into the intima. The proliferation is followed by an increase in connective tissue matrix, which results in the thickening of the medial and intimal layers. Specifically, previous studies (18, 26) in dogs have shown that an anastomotic occlusion occurs in venous retroperfusion due to fibrous proliferation. There may be species differences, however, because Chiu and Mulder (4) did not observe venous obliteration in the sheep model.

Occlusion of the LAD vein resulted in a significant increase in pressure and hence circumferential stress, which induces structural changes of the venous wall, such as neointimal hyperplasia and wall hypertrophy. At a FLP of ~0, the LAD vein is partially tethered by the myocardium. If we assume that the tethering is negligible, we can use Laplace’s equation to approximate the circumferential stress. Laplace’s equation states that the mean circumferential stress in the vessel wall is directly proportional to the blood pressure and inversely proportional to the vessel thickness-to-radius ratio. Therefore, an increase in blood pressure causes an increase in the mean circumferential stress. In pure hypertension, it is expected that the increase in wall thickness-to-radius ratio will occur in proportion to the increase in pressure, such that the circumferential stress is normalized (19, 23–25). This is in agreement with our present findings for the inward and hypertrophic remodeling of the LAD vein.

Recently, Hayashi et al. (9) have examined in rabbits the biomechanical response of the femoral vein to a chronic elevation of blood pressure. In response to a constriction of the iliac vein, they found that the femoral vein remodels to pres-
functional arterializations of the venous vessels. It is well known that fixation and histological processing of tissues, such as dehydration, embedding, sectioning, and staining, can cause dimensional changes and tissue distortion. In the present study, we used a fixation and tissue processing procedure to avoid these issues. However, the effect of fixation on venous remodeling is a task for future investigations.

Possible mechanism of remodeling. Among the many molecules implicated in vascular remodeling is tissue-type transglutaminase (tTG). tTG is an enzyme that has many functions, among which is the introduction of isopeptide linkages within a single polypeptide or between different polypeptides. Recently, Bakker et al. (1) found that tTG plays an important role in remodeling of small arteries. Their in vivo data showed that inward remodeling observed with low blood flow may be reversed by inhibiting the effects of the enzyme. Their in vitro experiments also showed that exogenous tTG induced inward remodeling on vessels pressurized at 60 mmHg. Langille and Dajnowiec (15) underscore the relationship between tTG and inward arterial remodeling.

Critique of methods. Laplace’s equation is only an approximation, because the mean circumferential stress is likely to be reduced due to the myocardial support (8, 27). Furthermore, because the vessel is partially tethered (posterior but not anterior), we expect the stress distribution in the wall to vary along the circumference. The computation of the local stress distribution in a partially or fully tethered vessel and the detailed relation of the stress to the remodeling pattern remains a task for future investigations.

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. Previously, Gross et al. (7) have shown that prior venous ligation reduces the mortality rate from subsequent coronary ligation. This is consistent with the well-known clinical observation that patients with right heart failure, and hence elevated coronary sinus pressure, are less prone to anginal pain.

The venous ligation changes not only local blood pressure but also blood flow. Although it is very difficult to measure blood flow in the epicardial veins because they are easily collapsible, it is reasonable to expect that the flow is decreased, especially near the ligation where there may be flow stagnation. The degree of flow reduction depends on the presence of bifurcations near the ligation that allows venting of flow. The adaptation of vessel lumen to changes in flow is in line with the constant wall shear stress hypothesis (11, 16, 17). Ultimately, the pattern of remodeling will depend on the degree of changes in both pressure and flow. Bakker et al. (2) found that the rat cremaster arterioles developed inward remodeling at zero flow and physiological pressure, whereas they did not observe remodeling at low pressure (3).

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dure that was recently shown to produce minimal distortion (5). In coronary arteries, it was found that the differences in dimensions between the histological sections and the fresh tissues to be within ~10% (5). The difference between the sham-operated and experimental tissue should be unaffected, however, because the same procedure was used in both groups.

In summary, the present study systematically characterizes the structural remodeling of the LAD vein at an intermediate pressure (between arterial and venous values). Our data show that the remodeling is nonuniform circumferentially and axially. This nonuniformity stems from the nonuniformity of the intramural stress field, which is dictated by the degree of radial myocardial constraint. In the venous vessels that are completely surrounded by the myocardium, the remodeling is circumferentially uniform as is the increase in wall thickness. The venous vessel is transformed from a thin-walled, large lumen vessel to a thicker-walled, smaller lumen vessel as depicted in Fig. 12. Future studies are needed where the prearterialized LAD veins are exposed to arterial pressure and flow, such as during coronary retroperfusion. With such studies we would expect further arterializations of the venous system in response to elevated pressure and expansive enlargement of the vessel lumens due to increased flow. Such observations and considerations are important for designing a therapeutic rationale for the nourishment of ischemic myocardium through venous retroperfusion.

ACKNOWLEDGMENTS

We thank Carlos Linares and Hans Mooij for excellent technical assistance.

GRANTS

This research was supported in part by the National Heart, Lung, and Blood Institute Grant 2-R01-HL-055554–06, the American Heart Association Grant-in-Aid 0555058Y, and TransVascular, Inc. (Menlo Park, CA).

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