Remodeling of Small Intramyocardial Coronary Arteries Distal to a Severe Epicardial Coronary Artery Stenosis

Huashan Hong, Sergei Aksenov, Xiaoming Guan, John T. Fallon, David Waters, Chunguang Chen

Objective—Impaired coronary blood flow (CF) or flow reserve with incomplete and delayed recovery of left ventricular (LV) function after revascularization is common in severe ischemic LV dysfunction. The underlying mechanism is not fully known. We studied structural changes of small intramyocardial coronary arteries (SIMCAs) in a pig model of chronic coronary stenosis, testing the hypothesis that microvascular remodeling develops distally to a severe epicardial coronary artery stenosis.

Methods and Results—A total of 24 pigs were studied in 3 groups. Left anterior descending coronary stenosis was created to reduce CF by a mean of ≈30%, producing severe regional systolic dysfunction without infarction. The stenosis was maintained for 7 days in 6 pigs (Group 1) and for 4 weeks in 12 pigs (Group 2). The control group (Group 3) consisted of 6 pigs with the same surgical procedures but without stenosis. The wall thickness (WTa) and lumen (L) diameter of SIMCA were measured, and the ratio of WTa/L and lumen area/total vessel area (% lumen) were calculated. The composition of the arterial wall was studied with cell proliferation markers Ki67 and BrdU. The immediate reduction in CF after the creation of the stenosis was similar in both study groups, but after the first week, CF decreased significantly (P<0.05) when the stenosis was maintained (group 2). The left anterior descending stenosis caused regional LV dysfunction in all pigs (groups 1 and 2). After 4 weeks of stenosis with chronic myocardial hibernation (group 2), but not after 1 week (group 1), WTa and WTa/L increased and L decreased significantly in the chronic hibernating region located distally to the stenosis, compared with both the control (group 3) and the normal region in the same pig. The mean % lumen of SIMCA per pig correlated with the CF reduction (r=0.92, P<0.001) and with myocardial fibrosis (r=0.82, P<0.01) in the 4-week stenosis group. Ki67- and BrdU-positive cells were increased in the wall of SIMCA in Group 1 and 2 compared with the control group (P<0.01 for each). The proliferated cells were stained positively with smooth muscle α-actin antibody.

Conclusion—In the chronic ischemic, hibernating myocardial region distal to a flow-limiting epicardial coronary stenosis, the small intramyocardial coronary arteries undergo remodeling, with an increase in wall thickness and a decrease in lumen. These structural changes may further restrict blood flow to ischemic myocardium and may account for the pathophyslogic impairment of CF or flow reserve after revascularization, which leads to delayed or incomplete recovery of myocardial function. (Arterioscler Thromb Vasc Biol. 2002;22:2059-2065.)

Key Words: coronary disease ■ ischemia ■ hibernation ■ microvascular disease

Impaired coronary flow reserve, reduced blood flow, and even no-reflow have been demonstrated after revascularization in dysfunctional myocardial regions that had been chronically ischemic or hibernating. This factor may contribute to a delayed, incomplete, or even absent functional recovery after revascularization. The mechanism accounting for the no-reflow or delayed-reflow phenomenon has been attributed to endothelial dysfunction, competitive flow from collaterals, distal embolization, or destruction of the microvasculature. Recent data suggest that chronic adaptive changes develop in the microvascular system distal to epicardial coronary stenosis or occlusion. However, information is limited regarding the arterial structural basis of the impaired flow after revascularization in chronic dysfunctional left ventricular (LV) regions. In one patient who died soon after bypass surgery, extensive thickening and lumen narrowing of small intramyocardial coronary arteries (SIMCAs) distal to an epicardial coronary stenosis was found to be responsible. A functional study of small vessels distal to coronary artery stenosis has suggested enhanced α-adrenergic contraction of the arterial wall and decreased nitric oxide synthesis. Decreased flow with reduced shear stress and decreased nitric oxide production in a low flow state in the distal vessel may lead to structural remodeling of the vascular...
wall. Therefore, using our previously established pig model of chronic coronary stenosis with ischemic, hibernating myocardium,3,11–13 we examined wall structures and lumen size of SIMCAs distal to a severe epicardial coronary stenosis, testing the hypothesis that SIMCAs undergo structural remodeling, which may further restrict flow to the hypoperfused myocardium and contribute to the reduced flow or impaired flow reserve after revascularization.

Methods

Animal Preparation

The Institutional Animal Care Committee at Newark Beth Israel Medical Center approved the study protocol. A proximal left descending (LAD) coronary stenosis was created as previously described, with modifications.3,11–13 Briefly, under general anesthesia and midline thoracotomy, the proximal LAD before its first diagonal branch was dissected over 1 to 2 cm to implant a coronary flowmeter probe (Transonic Inc, Ithaca, NY) and a hydraulic occluder. Transducer wires and tubing attaching to the hydraulic occluder were tunneled subcutaneously to the dorsal neck surface, exteriorized, and attached to the skin for monitoring LAD coronary flow (CF) and for later creation of the LAD stenosis.

After the pigs recovered from surgery (3 to 6 days), a severe LAD stenosis was created by gradually filling the hydraulic occluder with saline through a connecting tube to reduce resting LAD flow by 30% and produce regional LV dysfunction without causing infarction, which is consistent with myocardial hibernation. CF was monitored and measured by the implanted flowmeter at baseline during the first 60 minutes and at 24 hours after stenosis placement twice daily for the first week and then daily. In our previous experience, CF tends to increase during first 3 days of stenosis with the hydraulic occluder but remains relatively stable thereafter. Accordingly, the occluder was adjusted and monitored for 30 minutes daily during the first 3 days to maintain a relatively stable flow reduction of 30% under the same conscious, stable conditions with a similar heart rate and blood pressure. Variation of resting CF within ±10% was allowed. Measurements of CF under conscious conditions were used for analysis in this study.

Experiment Protocol

Three groups of 24 pigs total were studied. CF was reduced by 30% from baseline, and the reduction was maintained for 1 week in 6 pigs (group 1) and 4 weeks in 12 pigs (group 2). The same surgical procedures as in group 1 and 2 were performed without creating the LAD stenosis in 6 control pigs (group 3). Morphine (10 mg) or buprenorphine (0.05 to 0.1 mg/kg) was given intravenously immediately after placement of the LAD stenosis and during adjustment of coronary stenosis. Aspirin (325 mg) and intravenous heparin (20 000 IU/24 hours) were given postoperatively to prevent thrombotic coronary occlusion.

At the end of the experiment, pigs were euthanized, and 15 mL of 10% potassium chloride was given intravenously to arrest the heart in diastole. The heart was removed, and coronary vessels were flushed vigorously with iced physiological saline until the effluent was clear of blood. Next, after the hydraulic occluder was deflated to release the LAD coronary stenosis, the coronary vessels were cannulated and perfused with 10% formalin under a constant pressure of 80 mm Hg for 15 minutes.

Myocardial Blood Flow (MBF)

Myocardia that were perfused by the LAD were dissected and separated from normal regions and weighed. MBF was then calculated by dividing LAD flow by regional myocardial mass and expressed as mL/min/g of the wet tissue. To assess the collateral flow in the ischemic region, we performed myocardial contrast echocardiogram using mechanical index of 0.4 to 0.7, intermittent imaging (2:1), and bolus injection (0.3 mL) of Optison (human albumin microspheres with octafluoropropane formulation) into a peripheral vein during a brief (2 minutes) occlusion of LAD in 4 pigs at 4-week LAD stenosis (group 2). During the brief LAD occlusion, opacification or appearance of microbubbles in the ischemic region after the Optison injection was considered the presence of collateral flow. If there was no opacification, this was deemed the absence of collateral flow.

Echocardiographic Measurements

During the open-chest surgical procedure, right chest ribs were partially removed to avoid interference and to obtain a high-quality, transthoracic, two-dimensional echocardiogram for the evaluation of regional wall thickening, LV dimensions, volume, and ejection fraction as described previously.8

Morphometry of Intramyocardial Coronary Arteries

All LV tissues were fixed with 10% formalin, embedded in paraffin, sectioned at 5-μm intervals and stained with hematoxylin-eosin, trichrome, and Elastica von Gieson. Histological slides were evaluated at a magnification ×200 with a computer-integrated digital microscopic system (Image-Pro Plus, Silver Spring, MD). Consecutive microscopic fields were chosen for analysis using a predefined geometrical search pattern. All SIMCAs in the field were evaluated. External or outer diameters, excluding adventitia, internal, or lumen diameter and wall thickness (intima and media only), of SIMCAs were measured along the short axis of each vessel.14 The ratio of arterial wall thickness/lumen (WTa/L) was calculated. According to the size of the outer diameter, SIMCAs were divided into six categories: 10 to 30 μmol/L, 31 to 50 μmol/L, 51 to 70 μmol/L, 71 to 90 μmol/L, 91 to 120 μmol/L, and >120 μmol/L. The lumen long-axis dimension was also obtained to determine the degree of obliquity with which the vessel had been cut. Vessels with a long-to-short axis ratio of >1.5, indicating oblique cutting or inadequate pressure fixation of the samples, were excluded.15,16 The outer area (the outer border of the middle layer) of the vessel and lumen area (the inner border of the intimae) of the vessel were also measured; the ratio of internal lumen area and external arterial area was defined as percentage of luminal area (% lumen area = lumen area/external area of the artery including lumen area and arterial wall area) and was used as an index for normalization of lumen size by external vessel size to evaluate the degree of luminal narrowing of SIMCAs.15 Every vessel of each tissue section was counted, with the exception of those vessels of 10 μm or less and greater than 400 μm of outer diameter. Veins were excluded.

Analysis of Myocardial Fibrosis

The same slides were also used for morphometric analysis of myocardial fibrous connective tissue in myocardial hibernating regions and normal regions. The extent of fibrosis was quantitatively measured as described previously.8 The terms of segmental fibrosis, replacement fibrosis, and interstitial fibrosis that were described by Beltrami et al17 were applied in this study. The presence of segmental fibrosis, defined by fibrosis of more than 1 cm², was considered to be consistent with infarction.17

Immunohistochemistry of Cell Proliferation Markers (Ki-67 and BrdU) and α-Actin of Vascular Smooth Muscle Cells (VSMCs)

To determine whether cell proliferation was involved in SIMCA remodeling in chronic hibernation myocardium, at least 5 myocardial tissue sections from apex to base of the LV for each pig were stained with Ki-67, a monoclonal antibody to a nonhistone nuclear protein found in all phases of the cell cycle except G0.18 To confirm cell proliferation in SIMCAs, a cell cycle S-phase marker, BrdU (5'-bromo-2'-deoxyuridine, ICN) of 30 mg/kg was also given intramuscularly at 12 hours and 36 hours after creating proximal LAD coronary stenosis.19 (For detailed methods for Ki-67 and BrdU, see online version of this article, which can be accessed at http://atvb.ahajournals.org).
Hemodynamics, MBF, and Myocardial Dysfunction

On average, MBF was significantly reduced in group 1 and group 2 (P<0.01 each, Table 1). MBF was reduced significantly at 4 weeks in group 2 in comparison with the flow at 7 days (P<0.01, Table 1). No significant collateral flow or contrast opacification of myocardium was observed in the ischemic region using contrast echocardiography. Mean blood pressure did not change significantly in all groups (83±10 mm Hg at baseline, 82±11 mm Hg immediately after stenosis, and 85±11 mm Hg or 84±10 mm Hg at 7 days or at 4 weeks after stenosis; P>0.05). Heart rate was slightly higher after LAD stenosis when data from all groups were pooled (117±19 beats/min at baseline, 134±18 beats/min immediately after stenosis, and 131±21 beats/min at 7 days or 4 weeks after stenosis, P<0.05 each compared with baseline). Significant global (EF) and regional (WT) LV dysfunction developed in the pigs after LAD stenosis was created (Table 1). Left ventricular end-diastolic volume and LV wall mass increased significantly in the experimental groups, as shown in Table 1.

Results

Of the total of 24 pigs, 22 pigs were alive at the end of the study. Two pigs from group 2 died and were excluded from the analysis. An additional pig in group 2 exhibited no CF with a complete LAD occlusion as was detected by the implanted flowmeter. The pig was found to have transmural ischemic region using contrast echocardiography. Mean blood pressure did not change significantly in all groups (83±10 mm Hg at baseline, 82±11 mm Hg immediately after stenosis, and 85±11 mm Hg or 84±10 mm Hg at 7 days or at 4 weeks after stenosis; P>0.05). Heart rate was slightly higher after LAD stenosis when data from all groups were pooled (117±19 beats/min at baseline, 134±18 beats/min immediately after stenosis, and 131±21 beats/min at 7 days or 4 weeks after stenosis, P<0.05 each compared with baseline). Significant global (EF) and regional (WT) LV dysfunction developed in the pigs after LAD stenosis was created (Table 1). Left ventricular end-diastolic volume and LV wall mass increased significantly in the experimental groups, as shown in Table 1.

TABLE 1. Coronary Flow and LV Dimensions and Function in Experimental Groups

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>7-Day LAD Stenosis</th>
<th>4-Week LAD Stenosis</th>
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<tr>
<td></td>
<td>Group 1</td>
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<td>Group 1</td>
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<td>Group 2</td>
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<td>Group 2</td>
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<td>MBF, mL · min⁻¹ · g⁻¹</td>
<td>1.14±0.20</td>
<td>1.21±0.22</td>
<td>0.76±0.14*</td>
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<td>0.84±0.12*</td>
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<td>0.74±0.21*</td>
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<tr>
<td>Anterior WT, %</td>
<td>39±1</td>
<td>38±5</td>
<td>11±5*</td>
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<td></td>
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<td>13±4*</td>
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<td>9±4*</td>
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<td>Inferior WT, %</td>
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<td>40±3</td>
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<td>43±10†</td>
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<td>LV EF, %</td>
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<td>53±4</td>
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<td>29±9*</td>
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<td>LVEDV, mL</td>
<td>74±13</td>
<td>86±9</td>
<td>98±16*</td>
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<td>148±19*</td>
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<tr>
<td>LV mass, g</td>
<td>75±12</td>
<td>79±9</td>
<td>91±15†</td>
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<td>93±11†</td>
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<td>135±10*</td>
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LVEDV indicates left ventricular end-diastolic volume; LV mass, Left ventricular wall muscle mass; Group 1, 7-day LAD stenosis; Group 2, 4-week LAD stenosis.

*P<0.01 compared with baseline.
†P<0.05 compared with baseline.

Changes in SIMCA Distal to Epicardial Coronary Stenosis

Compared with the normal region of the same pig or pigs of the control group (Figure, panel A), a significantly thickened arterial wall with reduced lumen size of SIMCA was observed in the ischemic, hibernating myocardial region (Figure, panels B and C). There was no change in arterial wall thickness or lumen size in the normal region of group 1 and group 2 compared with the control group (P=NS). The thickened arterial wall was mainly composed of increased cells in the intimal layer with a mild increase of amorphous matrix, indicating intimal hyperplasia of SIMCA (Figure, panel D) in the 4-week hibernating myocardial region distal to a severe epicardial coronary disease. The degree of the intimal hyperplasia varied from mild, with minimal encroachment of the lumen (Figure, panel B), to severe with subtotal occlusion of the lumen (Figure, panel C). The increased cells in the inner and middle layers of the arterial wall in the hibernating region distal to a severe epicardial coronary stenosis were stained positively with ki-67 antibody and BrdU antibody, indicating cell proliferation (see online Figure I, which can be accessed at http://atvb.ahajournals.org). No significant change in arterial wall thickness was found in group 1 with 7-day LAD stenosis. However, Ki-67–positive cells were significantly increased in arterial wall in 7-day LAD stenosis (group 1, 2.6±0.5%, P<0.05) and in 4-week LAD stenosis (group 2, 5.8±0.5%, P<0.01) compared with that of Group 3 (0.9±0.4%). Similarly, BrdU-positive cells (32.7±2.1%) were significantly increased in the arterial wall of SIMCA in the 4-week hibernating myocardial region (group 2; see online Figure ID) compared with 5.8±2.1% (P<0.01) for BrdU-positive cells in the arterial wall of SIMCA of group 3 or with 6.1±2.0% (P<0.01) for BrdU-positive cells in the arterial wall of SIMCA in the normal myocardial region of the same group (group 2). Proliferative cells in the thickened arterial wall were positively stained by α-actin antibody, indicating the proliferation of VSMCs (see online Figure II, which can be accessed at http://atvb.ahajournals.org).

Quantitative evaluation of changes in the WTa and the ratio of WTa/L of SIMCA in normal and hibernating region are summarized in Table 2. In normal regions (the inferior region), there was no statistically significant difference in...
WTa and WTa/L of intramural coronary arteries between experimental groups with LAD stenosis and the control group. There was no significant increase in WTa or WTa/L ratio of SIMCA in group 1. WTa and WTa/L increased significantly \((P<0.01)\) for all SIMCAs of different sizes in the ischemic, hibernating region of group 2 after 4-week LAD stenosis compared with that of normal regions of the same group of pigs or compared with those vessels in the LAD region of the control group. The average arterial WTa of SIMCA in the 4-week hibernating region was \(24.3 \pm 9.24 \mu m\) and was significantly greater than that \((14.37 \pm 6.35 \mu m, P<0.01)\) of SIMCA in the normal region of the same group (group 2) with an average increase of 40.2%. The increase in WTa/L was more severe in smaller vessels of \(50 \mu m\) or less than those of greater than \(50 \mu m\) \((P<0.05)\). The abnormal SIMCA were focally and heterogeneously distributed and were found in areas with or without significant interstitial fibrosis in the hibernating myocardial region. Normal and abnormal vessels were observed adjacent to each other. Because of the heterogeneous nature of changes in SIMCAs, \% SIMCA with abnormal WTa/L (\(>\)mean \(\pm 2\ SD\) of normal value derived from control group) was calculated for each group. An abnormal WTa/L was present in 54\% of SIMCAs in group 2, in 4.2\% in group 1, and in 3.9\% in group 3. The increase in arterial wall thickness with abnormal WTa/L was randomly distributed in both the subendocardial and subepicardial regions. Slightly more vessels were affected in the subendocardial region than in the subepicardial region (63\% versus 45\%, respectively), but the difference did not reach a statistical significant level \((P>0.05)\).

The \% lumen area of SIMCAs in the ischemic region was \(17 \pm 15\%\) in group 2 of 4-week hibernating myocardium and was significantly smaller than in the same region of the control group \((38 \pm 13\%, P<0.01)\) or that of Group 1 with 7-day hibernating myocardium \((37 \pm 10\%, P<0.01, \text{Table 2})\). The \% arterial lumen of SIMCAs in the normal region (the inferior wall) of group 2 was \(34 \pm 11\%\) and was slightly smaller than that in the same region of the control group \((40 \pm 13\%), but the difference did not reach statistical significance \((P=\text{NS})\). There was no difference in the arterial lumen of the normal region between group 1 and the control group \((P=\text{NS})\).

**Myocardial Fibrosis and Microvascular Disease**

Interstitial, perivascular, and replacement fibrosis were found in all pigs in groups 1 and 2. Segmental fibrosis of more than 1 \(cm^2\) defined as patchy infarction was found in 2 pigs in group 2. The content of interstitial and replacement fibrosis was significantly increased in the 4-week ischemic, hibernating myocardium \((12.5 \pm 2.5\%)\) of group 2 compared with that \((2.1 \pm 1.0\%)\) of the control group \((P<0.01)\). The fibrosis was more prominent in subendocardial region \((19 \pm 5\%)\) than in the subepicardial region \((6 \pm 3\%, P<0.01)\). A less severe tissue showed that intermittent elastic lamina (black line) separated the proliferated intimal from the medial layer and the proliferated cells in the intimal layer were stained with anti-\(\alpha\)-actin antibody of smooth muscle the same as the smooth muscle cells in the medial layer. (Bar=50 \(\mu m\)).
Group 1 Collagen deposition in the walls of SIMCAs was also positively stained by markers Ki67 and BrdU. The proliferated cells stained Ki67, indicating the proliferation of VSMCs, and BrdU-positive cells than that of ki-67-positive cells in the arterial wall because BrdU was injected in the early stage at the second and third day of the LAD stenosis, representing an accumulative index of proliferated cells over weeks, whereas ki-67 was prepared only at the end of study, representing cells at their proliferative stage at a specific time point. Accumulatively, there was 32.7% increase in VSMCs in the arterial wall of the SIMCA in the ischemic region over a period of 4 weeks. The increase in collagen and hypertrophy of VSMCs, may have resulted in a marked increase in regional wall stress in the ischemic, hibernating region, which leads to an increase in regional myocardial intramural pressure that exerts on intramyocardial coronary arteries. In patients with hypertension the increase in collagen and hypertrophy of VSMCs, may have resulted in a marked increase in regional wall stress in the ischemic, hibernating region, which leads to an increase in regional myocardial intramural pressure that exerts on intramyocardial coronary arteries. In patients with hypertension increase of fibrosis was found in group 1 (6.7±2.4%) than in group 2 (P<0.01). There was no difference in fibrosis in normal regions between the control (2.1±1.2%) and groups of LAD stenosis (2.7±1.4%). The % arterial lumen of SIMCA in 4-week hibernating myocardium of group 2 correlated significantly with the reduction (% of MBF at the end of 4 weeks of LAD stenosis (r=0.92, P<0.001) and myocardial fibrosis (r=0.82, P<0.01). Neither heart rate nor blood pressure correlated with % lumen of SIMCA (P=NS).

**Discussion**

This study demonstrated that significant wall thickening and lumen narrowing of SIMCA developed distally to a severe, flow-limiting LAD stenosis in the ischemic, hibernating myocardial region over a period of 4 weeks. The increase in wall thickness and decrease in lumen of SIMCAs were found in all different sizes (external diameter of 10 to 400 \( \mu \)m) of intramyocardial coronary arteries, but the increase in ratio of arterial wall thickness and lumen diameter were more profound in smaller vessels. The increase in arterial wall thickness was proliferative, as indicated by proliferative cell markers Ki67 and BrdU. The proliferated cells stained positively by \( \alpha \)-actin, indicating the proliferation of VSMCs. Collagen deposition in the walls of SIMCAs was also modestly increased. These changes are similar to proliferative process after arterial injuries.\(^{20-22}\) Ki-67–positive cells increased 6 times or more in the walls of SIMCAs (5.8%) distal to the epicardial LAD stenosis in hibernating myocardial region compared with those of the control group (0.9%). A similar, more than 6-fold increase was observed in BrdU-positive cells (32.7%) in the arterial wall of 4-week hibernating myocardium (control group, 5.8%). Different cell phases are labeled with ki-67 compared with the cell phase labeled by BrdU. Ki67 is a nonhistone nuclear protein that is found in late G1, S, G2, and M phases of the cell cycle, whereas BrdU, a halogenated pyrimidine analog of thymidine, is incorporated into the DNA of proliferating cells and identifies only S-phase of cell cycle. We observed a higher percentage of BrdU-positive cells than that of ki-67–positive cells in the arterial wall because BrdU was injected in the early stage at the second and third day of the LAD stenosis, representing an accumulative index of proliferated cells over weeks, whereas ki-67 was prepared only at the end of study, representing cells at their proliferative stage at a specific time point. Accumulatively, there was 32.7% increase in VSMCs in the arterial wall of the SIMCA in the ischemic region over a 4-week period. In the same period, there was 40.2% increase in arterial wall thickness of SIMCAs in the ischemic region. Therefore, the increase in VSMCs accounted for about 80% of the increase in arterial wall thickness, assuming that the diameter of VSMC was unchanged. Other factors, such as increase in collagen and hypertrophy of VSMCs, may have been contributed to the remaining 20% of the increase in the arterial wall thickness. Whether and to which extent of apoptosis of smooth muscle cell were involved in the microvascular remodeling process are not known from the data of this study and would require further investigation.

Although wall thickening with lumen narrowing of SIMCA did not occur in 7-day hibernating myocardial regions distal to the severe epicardial coronary stenosis, there was a high percentage of Ki-67–positive smooth muscle cells in the SIMCA of 1-week hibernating regions, indicating that VSMCs in the SIMCAs have already entered into proliferative phases in the first week after initiation of epicardial coronary stenosis.
or aortic valve disease, the extravascular pressure on SIMCAs, but not coronary perfusion pressure, correlates with arterial wall thickening and lumen narrowing of SIMCAs.\textsuperscript{17} Second, recent studies of arterial remodeling in epicardial coronary arteries in the content of de novo atherosclerosis and restenosis have demonstrated that increased plaque growth and proliferation of VSMC or fibroblast is more prominent downstream of epicardial coronary artery stenosis than at the proximal segment of the arteries.\textsuperscript{24} Changes in the fluid mechanical force from altered blood flow (shear stress) have direct effects on artery wall structure during growth and development\textsuperscript{26,27} and on the progression of atherosclerosis and intimal hyperplasia.\textsuperscript{30} An increase in shear stress or blood flow results in outward remodeling, which increases vessel size. In contrast, inward remodeling with reduced vessel size occurs in low-flow states with reduced shear stress. Flow-limiting proximal LAD stenosis created in this study reduced shear stress in the distal vessels. Reduced shear stress accentuates production of mitogenic and fibrogenic growth factors, such as platelet-derived growth factor and transforming growth factors.\textsuperscript{28} Low blood flow or shear stress of coronary artery also leads to underexpression of growth inhibitors, such as nitric oxide.\textsuperscript{29} Nitric oxide, in addition to its vasodilator properties, has been demonstrated to inhibit smooth muscle cell growth, intimal hyperplasia, platelet adhesion, and platelet-derived growth factor expression.\textsuperscript{30} Ito et al\textsuperscript{31} demonstrated that in pigs without epicardial coronary stenosis, chronic (2-week) inhibition of endothelium-derived nitric oxide synthesis by local infusion of N\textsuperscript{\textsubscript{\textit{O}}}-nitro-L-arginine methyl ester causes coronary microvascular structural changes with medial wall thickening in small intramyocardial coronary arteries, which is similar to the microvascular changes observed in our study but to a lesser extent. This suggests that intrinsic nitric oxide is not only important for microvascular function but also for its structural integrity. Reduced production of nitric oxide during ischemia may have contributed to the structural remodeling of microvascular system. Third, the myocardial ischemia that occurs in the hibernating region during daily activities impairs endothelial function of downstream intramural coronary arteries. Endothelial dysfunction reduces the production of growth inhibitors and vasodilators, such as nitric oxide. Finally, increased productions of angiotensin II, endothelin, and catecholamines may not only contribute to myocardial remodeling but may also lead to VSMC hypertrophy and proliferation during myocardial ischemia and LV remodeling. In fact, preliminary study from this laboratory showed a significant reduction of intimal hyperplasia in SIMCAs by attenuating ischemia and LV remodeling with a \textit{\textbeta}-blocker (oral metoprolol of 100 to 200 mg/day) in pigs with 4-week LAD stenosis.\textsuperscript{32}

Microvascular Remodeling, CF Reduction, and Degree of Myocardial Fibrosis

The initial mild reduction (\textasciitilde30\%) of resting CF caused a modest degree of myocardial fibrosis at 7 days after the creation of the epicardial coronary stenosis. The fibrosis progressively increased over 4 weeks, even though the degree of the epicardial stenosis was not changed. The mechanism of the progressive increase in fibrosis in the ischemic region over 4 weeks is not fully understood.\textsuperscript{3} This study demonstrated that the severity of CF reduction correlated significantly with the severity of wall thickening and lumen reduction of SIMCAs at the end of 4 weeks of LAD stenosis and that the degree of myocardial fibrosis was related to the degree of CF reduction in the chronic ischemic myocardial region. This suggests that small vessel disease distal to a severe coronary artery disease contributes to a further myocardial blood flow reduction distal to a fixed chronic epicardial coronary stenosis and thus, caused an increased degree of myocardial fibrosis. Furthermore, the fact that the arterial wall thickening with an intimal hyperplasia and narrowed lumen was found in ischemic regions that had not developed significant fibrosis (Figure, panel B) strongly suggests that reduced arterial lumen by vascular smooth muscle proliferation was not merely the consequence of myocardial fibrosis or scarring. Arterial wall thickening with lumen narrowing increases vascular resistance and further reduces blood flow to hibernating myocardium, producing ischemia. Further studies are required to determine whether a decreasing CF secondary to progressive small arterial wall thickening and lumen narrowing distal to a fixed epicardial coronary stenosis may lead to an increase in myocardial fibrosis, deterioration of myocardial function, and progression of LV dilatation. LV dilatation further increases LV wall stress, which may, in turn, further increase oxygen consumption and myocardial ischemia, forming a vicious circle.

Limitations

In the current study, coronary artery stenosis was not released to test CF or flow reserve without epicardial coronary stenosis. Two pigs had segmental fibrosis and were considered to have myocardial infarction coexisting with hibernating myocardium, which may have confounded the result of the study. However, the extent of vascular remodeling in the noninfarcted region of the hearts of these two pigs did not seem to be different from those of pigs without coexisting infarction. Furthermore, vessels in the infarcted region were excluded from the analysis. Reversibility of the hibernating myocardium in group 2 of 4-week hibernating myocardium was not studied. However, reversibility of the hibernating myocardium was confirmed by reperfusion in our previous study with this model.\textsuperscript{3} BrdU was injected only in the first week after coronary stenosis and more than one third of the cells in the vessel wall were stained positive. The percentage of BrdU-positive cells may have even been higher if BrdU was also injected during the second and third week after the coronary stenosis. No vessel dilator agent was used in this study before fixing the vessels, which may raise the concern of acute vessel tone- or low flow–related changes. However, there was no significant change in arterial wall thickness and lumen size of SIMCAs in the short-term coronary stenosis of 7 days in group 1 compared with the controls. This excluded the possibility that increased wall thickness and decreased arterial lumen in the SIMCA was secondary to variations in vessel tone–related to low flow–related change.

In summary, this study demonstrated that microvascular remodeling with significant arterial wall thickening and lumen narrowing developed distally to a chronic, flow-
limiting epicardial LAD stenosis in hibernating myocardial region over a 4-week period. Smooth muscle cell proliferation was found to be primarily responsible for the arterial wall thickening that encroached on the small arterial lumen. These changes in small intramyocardial coronary arteries were associated with further reduction of myocardial blood flow, deterioration of myocardial function, progression of ventricular dilatation, and an increase of myocardial fibrosis in hibernating myocardial region.

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