Compensatory growth of coronary arterioles in postinfarcted heart: regional differences in DNA synthesis and growth factor/receptor expression patterns

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Dedkov, Eduard I., Wei Zheng, and Robert J. Tomanek. Compensatory growth of coronary arterioles in postinfarcted heart: regional differences in DNA synthesis and growth factor/receptor expression patterns. Am J Physiol Heart Circ Physiol 291: H1686–H1693, 2006.First published May 19, 2006; doi:10.1152/ajpheart.00307.2006.—Previous studies have not addressed regional differences in adaptive arteriolar growth in the surviving left ventricular (LV) myocardium after infarction in appropriately aged animals, namely middle-aged or older. Accordingly, we examined the adaptive postinfarction growth of arterioles in two distinct regions, i.e., the LV free wall (LVFW) and septum, of middle-aged rats. We induced a myocardial infarction (MI) in 12-mo-old rats to analyze 1) protein expression in VEGF/Flt-1/Flk-1 and angiopoietin (Ang)-1/Ang-2/Tie-2 systems, 2) the arteriolar DNA synthesis, 3) the extent of the arteriolar bed, and 4) the alteration in minimal coronary vascular resistance. In both regions, arteriolar DNA synthesis was activated between days 4 and 7 after MI. Whereas in the LVFW the degree of DNA synthesis declined between days 11 and 14 post-MI, it continued to rise in the septum, and at day 14, the percentage of the arterioles undergoing DNA synthesis was comparable in the LVFW and the septum (9.7 ± 1.6 and 7 ± 2.1%, respectively). Arteriolar DNA synthesis was mainly associated with upregulation of Ang-2 and Tie-2 in both LV regions. Although wk after MI the arteriolar beds in the LVFW and the septum expanded to the size of sham-operated rats, this growth did not compensate for the greater minimal coronary vascular resistance in the former. Thus our findings suggest that 1) the dynamics in adaptive arteriolar growth were similar between the two regions, despite a delay in the septum; and 2) the perfusion deficit in post-MI rats cannot be accounted for by inadequate adaptive growth of arterioles.

myocardial infarction; arteriolar density; minimal coronary vascular resistance; angiogenic growth factors

THE FACT THAT CORONARY RESISTANCE vessels grow in the surviving myocardium during cardiac remodeling after a large myocardial infarction (MI) of the left ventricular (LV) free wall (LVFW) is well established (1, 5, 12–14). However, because our group (1) has shown that impaired maximal myocardial perfusion is not necessarily a consequence of inadequate anatomical growth of the arteriolar bed, it is imperative that both parameters be evaluated. Furthermore, because the results from the studies in which young or young-adult post-MI rats were used (4–10, 13, 21) could be complicated by the concurrent presence of both normal and adaptive vascular growth (13), the use of older, i.e., middle-aged or senescent rats, is more appropriate. Moreover, the adaptive modifications in postinfarcted hearts of middle-aged rats can provide a better correlate to human infarctions that occur primarily in middle-aged and senescent individuals.

Most important, a number of key issues regarding adaptive growth of the coronary arterioles in post-MI hearts remain unresolved. For instance, previous studies (12, 14, 19) focused on DNA activity in coronary capillaries but not arterioles. Furthermore, although the involvement of two main angiogenic growth factor/receptor regulatory systems, i.e., angiopoietin (Ang)-1/Ang-2/Tie-2 and VEGF/Flt-1/Flk-1, in MI-associated growth of coronary vasculature has been previously established (10, 11, 15, 16, 21), their temporal expression patterns during compensatory growth of coronary arterioles in the nonischemic LVFW and septum remain undetermined. The discrete analysis of these two LV regions is important because they have separate arterial systems (Fig. 1B) (17, 18) and underwent different degrees of post-MI diastolic wall stress (3) and hypertrophy (1, 7, 8, 21). All these regional peculiarities may affect the expression patterns in a unique manner. Therefore, our current study on post-MI adaptation of the resistance vasculature was based on three aims: 1) the delineation of the temporal pattern of protein expression in Ang-1/Ang-2/Tie-2 and VEGF/Flt-1/Flk-1 angiogenic regulatory systems; 2) the degree of compensatory arteriolar growth; and 3) the change in minimal coronary vascular resistance (MCVR) in two distinct LV regions, i.e., LVFW, excluding regions adjacent to the infarct, and the septum.

MATERIALS AND METHODS

All procedures were performed in accordance with the Guide for the Care and Use of Laboratory Animals, published by the National Institutes of Health (NIH Publication No. 85-23, Revised 1996) and were approved by the University of Iowa Animal Care and Use Committee.

Animals and experimental protocols. MI was induced in 12-mo-old male Sprague-Dawley rats (Harlan, Indianapolis, IN) under anesthesia with a mixture of ketamine (100 mg/kg ip) and xylazine (10 mg/kg ip) by left coronary artery ligation (Fig. 1B), as detailed previously (1, 10, 21).

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Maximal myocardial perfusion and calculation of MCVR per unit mass. The rats were anesthetized with a mixture of ketamine (100 mg/kg ip) and xylazine (10 mg/kg ip), and polyethylene catheters were inserted into the jugular vein, into both femoral arteries, and into the left ventricle via the right common carotid artery.

Maximal myocardial perfusion in the LVFW and septum was determined by using neutron-activated, stable isotope-labeled microspheres (BioPAL, Worcester, MA), as detailed previously (1, 10). Briefly, ~1 × 10⁶ microspheres were infused into the left ventricle after maximal vasodilatation via dipryidamole (6 mg·kg⁻¹·min⁻¹ for 8 min). Simultaneously, arterial reference blood samples were withdrawn from the left femoral artery for 2 min at a constant rate (0.2 ml/min), whereas the mean arterial pressure, i.e., perfusion pressure, was recorded via the right femoral artery. At the end of the experiment, tissue and reference blood samples were sent to the BioPhysics Assay Laboratory (BioPAL) for microsphere counting. Regional myocardial perfusion was computed as \( \frac{C_m}{C_r} \times Q_r \times 10^3 \text{ g} \) (in ml-min⁻¹·100 g⁻¹), where \( C_m \) is the number of microspheres per gram of myocardium, \( C_r \) is the number of microspheres in the reference blood sample, and \( Q_r \) is the withdrawal rate of the reference blood sample. To compute regional MCVR per 100 g, perfusion pressure was divided by corresponding maximal myocardial perfusion.

Tissue sampling and preparation. In group 1 (Fig. 1A), the hearts were excised, washed in phosphate-buffered saline, and then cut into parallel slices with a guillotine. The samples of myocardium from the LVFW and the septum (Fig. 1C) were quickly excised and frozen in liquid nitrogen.

In groups 2 and 3 (Fig. 1A), the hearts were arrested in diastole with 2% lidocaine, excised, and perfuse-fixed on a Langendorff apparatus under constant pressure (100 mmHg) with 4% paraformaldehyde in phosphate-buffered saline. The atria and the right ventricular free wall were removed, and the hearts were cut transversely into parallel slices with a guillotine. In the hearts with MI, the LV slices were digitized, and infarct size was estimated using Image-Pro Plus 5.1 software (Media Cybernetics, Silver Spring, MD), as detailed previously (1, 10, 21). Infarct size was expressed as a percentage of the LVFW. Additionally, in the third group of rats, the left ventricle, including septum, was weighted. From each heart, one midventricular slice was processed and embedded in paraffin. In the remaining LV slices, samples of myocardium from the LVFW and septum (Fig. 1C) were excised, weighted, and used for microsphere counting.

Immunohistochemistry and fluorescence microscopy. Transverse 8.0-μm-thick serial sections were cut from LV slices embedded in paraffin, deparaffinized, rehydrated, and double immunostained with a monocular Cy3-conjugated anti-SM α-actin antibody, clone 1A4 (Sigma) and a polyclonal anti-laminin antibody (Sigma), as detailed previously (1). The LV sections of the hearts from BrdU-involved experiments were double immunolabeled with a monoclonal Cy3-conjugated anti-SM α-actin antibody, clone 1A4 (Sigma) and a monoclonal anti-BrdU antibody (Chemicon International, Temecula, CA). These sections were counterstained with 4′,6-diamidino-2-phenylindole (DAPI, 10 mg/ml) to identify nuclei. Alexa Fluor 488 goat anti-rabbit IgG and goat anti-mouse IgG (Molecular Probes, Eugene, OR) were used for visualization of anti-laminin and anti-BrdU antibodies, respectively. Fluorescence images were captured on a computer using a Nikon Eclipse E-600 microscope, equipped with a Nikon digital DXM 1200 camera.

Quantitative morphometry and stereological analyses. Morphometric and stereological analyses were conducted on digitized images using Image-Pro Plus 5.1 software. Laminin-outlined profiles were used to estimate myocyte cross-sectional area, whereas SM α-actin positive profiles (5 to 54 μm in diameter) were used to calculate the arteriolar length and volume densities, as detailed previously (1). From each BrdU-labeled heart, we randomly selected 100 arterioles from the LVFW and 100 from the septum. The arterioles with one or more endothelial cell and/or SM cell nuclei that stained positively

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**Fig. 1.** Schematic illustrations. *A*: time course of experiments. *B*: arterial blood supply to left ventricular (LV) myocardium and level of coronary artery occlusion. *C*: regions of LV free wall (FW) and septum (S) used for evaluation. In rats from *group 1*, protein expressions of angiogenic growth factors and their receptors were assessed by Western blot (WB) analysis only at three specific post-myocardial infarction (MI) time points. In *group 2*, although rats were euthanized at same time points as in *group 1*, we estimated percentage of coronary arterioles, which were 5-bromo-2′-deoxyuridine (BrdU)-labeled during 72 h of BrdU infusion (between days 0, 3, 4, and 7, and 11 and 14 post-MI). Rats from *group 3* were used only for the 4-wk study. Time points used in our experiments were chosen to coincide with the majority of earlier published studies. In *B*, septal artery (SA) and left coronary artery (LCA), shown as two distinct branches of the left main coronary artery (LMCA), as described in Sprague-Dawley rats by Spadaro and colleagues (see Ref. 17), provide the independent arterial systems to the LVFW and S. 21). Three separate groups of rats were employed (Fig. 1A). In the first group, post-MI (*n* = 12) and sham-operated (*n* = 12) rats were euthanized on *days 3, 7,* and 14 after surgery, and the hearts were collected for Western blot analysis to assess the expression of angiogenic growth factors and their receptors. In the second group, starting on *days 0, 4*, or 11 after surgery, post-MI (*n* = 18) and sham-operated (*n* = 18) rats received 12.5 mg·kg⁻¹·day⁻¹ of 5-bromo-2′-deoxyuridine (BrdU; Sigma, St. Louis, MO) intraperitoneally for 72 h via ALZET osmotic pumps (Durect, Cupertino, CA). The hearts of these rats were analyzed by immunohistochemistry to determine the extent of BrdU labeling in smooth muscle (SM) and endothelial cells of coronary arterioles on *days 3, 7,* or 14 after MI. In the third group of post-MI (*n* = 8) and sham-operated (*n* = 12) rats, maximal myocardial perfusion was measured 4 wk after surgery, and the hearts were collected for morphological analysis. After LV sections were immunostained for SM α-actin, the size of the coronary arteriolar bed was determined by quantitative morphometry and stereological analysis.

The data from post-MI rats were included in the study only if the infarct size was between 50 to 75% of the LVFW. All data from the LVFW of post-MI rats were derived from tissue ~1.5–2 mm distal to the infarct edge (Fig. 1C).
with an anti-BrdU antibody were identified and counted. The final number of BrdU-labeled arterioles per each region was expressed as a fraction percentage of the total arterioles examined.

**Western blot analysis.** The analyses were performed as detailed previously (10, 21). Briefly, frozen samples were homogenized in a lysis buffer containing protease inhibitors. Protein concentrations were determined using the Bio-Rad Protein Assay (Hercules, CA). The protein extracts were subjected to SDS-PAGE under reducing conditions and were transferred to Hybond ECL nitrocellulose membranes (Amersham Biosciences, Piscataway, NJ). The membranes were incubated with VEGF (A-20), Flk-1 (Q-20), Flt-1 (C-17), and Tie-2 (C-20) polyclonal antibodies (Santa Cruz Biotechnology, Santa Cruz, CA); Ang-1 (Ang11-S) and Ang-2 (Ang21-S) polyclonal antibodies (Alpha Diagnostic International, San Antonio, TX); and GAPDH monoclonal antibody, clone 6C5 (Chemicon International). The primary antibodies were visualized with the corresponding IRDye-800-conjugated secondary antibodies, and membranes were analyzed by using the Odyssey Infrared Imaging System (LI-COR, Lincoln, NE). The intensity of protein expression was assessed by means of quantitative densitometry analysis and then normalized to corresponding GAPDH expression in each sample. The level of individual protein expression is expressed as fold change from the level in sham-operated rats.

**Statistical analysis.** Data are expressed as means ± SE. One-way ANOVA was used, followed by unpaired t-test to assess intergroup differences. P < 0.05 was selected to denote significant differences.

**RESULTS**

**Angiogenic growth factor and receptor protein expression.** The protein expression of VEGF, Flt-1 (VEGF receptor type 1), Flk-1 (VEGF receptor type 2), Ang-1, Ang-2, and Tie-2 receptor was assessed in the LVFW and the septum (Fig. 1C) 3, 7, and 14 days after MI. In both regions, the level of VEGF (Fig. 2) and Ang-1 (Fig. 3) expression remained relatively comparable during the 2 wk after MI to those of sham-operated rats. In contrast, the level of Ang-2 expression in the LVFW became significantly augmented between 3 and 7 days after MI, compared with sham-operated rats, and remained markedly elevated 1 wk later (Fig. 3). At the same time, an increase in the level of Ang-2 expression was also detected in the septum, but such an increase was delayed, compared with the LVFW, because the increase was noted only at day 14 after MI.

The levels of Flt-1 and Flk-1 expression in the LVFW, which were significantly elevated at day 3 after MI, decreased markedly at days 7 and 14 after MI (Fig. 2). At the same time, in the septum, although the level of Flt-1 expression remained unchanged over 2-wk of post-MI period, the level of Flk-1 expression showed a temporal pattern similar to that in LVFW, i.e., the initial rise in Flk-1 protein production around day 3 after MI, followed by a marked drop when compared to the level detected in the sham-operated rats.

The level of Tie-2 expression in the LVFW was significantly elevated during the first 2 wk after MI, with a peak at day 7 (Fig. 3). In the septum, the temporal pattern of Tie-2 expression was similar but of a lower magnitude compared with that in LVFW. Thus, despite a delay in the increase of the Ang-2 production in the septum, the overall pattern of protein expression of angiogenic growth factors and their receptors was similar between the two LV regions.

**Fig. 2.** VEGF, Flt-1, and Flk-1 protein expression in noninfarcted myocardium of the LVFW and S assessed at days 3 (or 3D), 7 (or 7D), and 14 (or 14D) after MI. Level of protein expression is shown as fold change relative to value detected in sham-operated rats (denoted as one). Values are means ± SE; n = 4 rats. **P < 0.01 vs. sham; † P < 0.05 vs. day 3 after MI.

**Fig. 3.** Ang-1, Ang-2, and Tie-2 protein expression in noninfarcted myocardium of the LVFW and S assessed at days 3, 7, and 14 after MI. Level of protein expression is shown as fold change relative to value detected in sham-operated rats (denoted as one). Values are means ± SE; n = 4 rats. * P < 0.05, ** P < 0.01, and *** P < 0.001 vs. sham; † P < 0.05 vs. day 3 after MI; £ P < 0.05 and §§ £ P < 0.01 vs. day 7 after MI.
DNA synthesis in coronary arterioles. As illustrated in Fig. 4, DNA synthesis in SM and endothelial cells of coronary arterioles is activated during the 2-wk period after MI. The percentage of coronary arterioles in which SM and/or endothelial cells were labeled with an anti-BrdU antibody became significantly higher in the LVFW as well as in the septum starting at day 7 after MI, compared with that in sham-operated rats and rats at day 3 after MI (Fig. 5A). Furthermore, 7 days after MI, the percentage of vessels with BrdU-labeled cells was significantly greater in the LVFW than that in the septum (13.2 ± 2.4% vs. 2.2 ± 0.9%, respectively, P < 0.001). Fourteen days after MI, the number of arterioles with BrdU-labeled cells was slightly reduced in the LVFW, but it increased in the septum (Fig. 5A). At this time point, the percentage of the labeled arterioles was comparable between the two regions (9.7 ± 1.6% in the free wall vs. 7 ± 2.1% in the septum), indicating that a potent growth response occurred in the arteriolar tree of the septum, but such response was delayed compared with that in LVFW. Because significant increases in BrdU labeling were noted only at days 7 and 14 after MI, the morphometric analysis of arterioles by various size (diameter) classes was limited to these two time points (Fig. 5B). The data indicate that growth occurred throughout the entire arteriolar tree in both LV regions and that it increased progressively with vessel diameter (Fig. 5B).

We next evaluated the arterioles in relation to the type of cells demonstrating DNA synthesis (Fig. 4, A–D). The BrdU labeling of SM and endothelial cells increased over time in a similar pattern in both the LVFW and septum (Fig. 6). However, the percentage of vessels with BrdU-labeled SM cells was always greater than those with BrdU-labeled endothelial cells. Because the number of coronary arterioles in which both type of cells were labeled was scant (Fig. 4, E and F), their effect on the final calculations was negligible.

Our data indicate that despite a delay in the rise of the number of growing arterioles within the septum, the overall pattern of arteriolar growth was comparable between the two LV regions, although the magnitude of such growth was always greater in the LVFW.

![Fig. 4. Representative images of double immunofluorescence tissue staining using anti-smooth muscle (SM) α-actin antibody (red) and anti-BrdU antibody (green) to identify coronary arterioles and cells undergoing DNA synthesis. In B, D, and F, nuclei are counterstained with 4',6-diamidino-2-phenylindole (DAPI; blue). In A and B (a large arteriole from LVFW at day 7 after MI), arrowheads indicate DNA synthetizing nuclei of endothelial (ET) cells, i.e., cells located on inner side of arteriole, and showed no immunostaining against SM α-actin (marker of SM cells). Because we perfuse-fixed the hearts, the presence of BrdU-labeled circulating cells inside arterioles is unlikely; bar = 20 μm. In C and D (middle-size arteriole from S at day 7 after MI), arrowhead indicates DNA synthetizing nucleus of endothelial (ET) cells, i.e., cells located on inner side of arteriole, and showed no immunostaining against SM α-actin (marker of SM cells). Because we perfuse-fixed the hearts, the presence of BrdU-labeled circulating cells inside arterioles is unlikely; bar = 20 μm. In E and F (small arteriole from LVFW at day 14 after MI), arrowheads indicate the DNA synthetizing nuclei of SM cells, and an asterisk is placed on the BrdU-positive nucleus of an endothelial cell. Arrow indicates BrdU-positive nucleus of interstitial cell; bar = 25 μm.](http://ajpheart.physiology.org/10.1152/ajpheart.00302.2006)
LV hypertrophy, MCVR, and size of coronary arteriolar bed. Four weeks after MI, the average infarct size was 63.7 ± 2.4% of the LVFW. Despite a significant infarct-associated loss of myocardial tissue, LV weight and LV weight-to-body weight ratio in post-MI rats (1074.2 ± 52.2 mg and 2.02 ± 0.06 mg/g, respectively) were similar to those of sham-operated rats (1191 ± 49.1 mg and 2.1 ± 0.1 mg/g, respectively), indicating that hypertrophy of the surviving myocardium fully compensated for the myocyte loss due to infarction. This growth was associated with a transverse enlargement of myocytes in the LVFW where their cross-sectional area increased by 46.4 ± 8.5% (P < 0.05) compared with that in sham-operated rats. At the same time, septal myocytes did not undergo noticeable hypertrophy (Table 1).

The size of the coronary arteriolar bed was assessed by means of arteriolar length and volume densities (Table 1). In the LVFW of post-MI hearts, the density of coronary arterioles was similar to that in sham-operated rats, indicating that compensatory growth of coronary arterioles approximated the magnitude of myocyte enlargement in this part of myocardium. At the same time, although there was no noticeable hypertrophic reaction in the septum of post-MI rats, volume density of the coronary arteriolar bed expanded significantly in this part of LV myocardium, i.e., it exceeded the arteriolar volume density of sham-operated rats by 24.8 ± 4.9% (P < 0.05). The evaluation of arteriolar length and volume densities in relation to vessel diameter revealed that in both LV regions most of the arteriolar growth occurred in the large arterioles (25–54 μm in diameter) (Fig. 7).

To detect whether the compensatory growth of coronary arterioles would fully rescue the maximal myocardial perfusion in the remaining LV myocardium of post-MI hearts, the MCVR per unit mass was estimated. Despite the fact that arteriolar density was comparable between post-MI and sham-operated rats, the MCVR per 100 g was markedly increased after MI, by 33.8 ± 7% (P < 0.01) in the LVFW and by 33.1 ± 7.3% (P < 0.01) in the septum (Table 1). This indicates that the surviving LV myocardium of post-MI hearts experienced a significant perfusion deficit, which is not related to the inadequate compensatory growth of coronary arterioles.

Thus, although the MCVR per unit mass was compromised in the LVFW and the septum of post-MI rats, the extent of coronary arteriolar beds in these two regions was similar to that of sham-operated rats. Therefore, the marked perfusion deficit detected in LV myocardium of post-MI hearts cannot be accounted for by inadequate compensatory growth of coronary arterioles.

Fig. 5. Quantitative analysis of coronary arterioles showing presence of BrdU-labeled SM and/or ET cells. A: percent labeling in sham-operated rats and rats at 3, 7, and 14 days after MI. LVFW (A and B, top) and S (A and B, bottom) are shown. B: percent labeling in various sized (diameter classes) arterioles at 7 and 14 days after MI. Number of BrdU-labeled arterioles per each diameter class was expressed as fraction percentage from total number of arterioles per corresponding class. Values are means ± SE; n = 6 rats. *P < 0.05 and **P < 0.01 vs. sham; †P < 0.05 and ††P < 0.01 vs. day 3 after MI; §P < 0.05 vs. day 7 after MI. B: *P < 0.05 and **P < 0.01 vs. day 7 after MI; †P < 0.05 and ††P < 0.01 vs. class of smallest arterioles (5–14 μm in diameter).

DISCUSSION

This study focused on middle-aged rats to provide data that reflect the stage of life in humans when MI frequently occurs.
Our findings document a potent adaptive arteriolar growth in the surviving nonischemic myocardium of both the LVFW and septum during post-MI cardiac remodeling. Such arteriolar growth, indicated by morphometric analysis and activation of DNA synthesis, was associated with a marked upregulation of Ang-2 and Tie-2 proteins. Interestingly, although protein up-regulation in two main angiogenic growth factor/receptor systems (Ang-1/Ang-2/Tie-2 and VEGF/Flt-1/Flk-1) and the increase in DNA synthesis in the cells of coronary arterioles were similar in the two LV regions, their onsets were delayed in the septum. Surprisingly, although 4 wk after MI-induced cardiac remodeling, the arteriolar beds in the surviving nonischemic myocardium expanded to the size of those in sham-operated rats (in the LVFW) or even in excess of those in the sham myocardium (in the septum). Surprisingly, although 4 wk after MI-induced cardiac remodeling, the arteriolar beds in the surviving nonischemic myocardium expanded to the size of those in sham-operated rats (in the LVFW) or even in excess of those in the sham-operated rats (in the septum), it did not compensate for the greater MCVR in both regions of the post-MI hearts.

Compensatory expansion of arteriolar bed after MI. Most studies (4 –9, 13) that reported insufficient growth of resistance vessels in the surviving LV myocardium of young or young-adult post-MI rats based their conclusions regarding adaptive vascular growth solely on maximal myocardial perfusion. These data appear to support the conclusion that the growth of resistance vessels failed to match the degree of cardiac hypertrophy, especially in the LVFW where hypertrophy of cardiac myocytes is the greatest (1, 4, 7–9). However, our current data on middle-aged post-MI rats indicated that although the MCVR was increased in LVFW, the growth of coronary arterioles (estimated by morphometry) approximated the degree of myocyte enlargement in that region. Surprisingly, the current study found an increase in MCVR in the septum, despite a lack of significant concentric cardiac myocyte enlargement. Kalkman and colleagues (7, 8) also reported a lack of hypertrophy in the septum of post-MI rats, but it was associated with normal perfusion. Because of the well-established fact that in young rats the myocardial vasculature continues normal growth (13), the best explanation of these disparate findings might be our use of older rats in which the coronary vasculature is growth quiescent. However, we detected an almost 25% increase in arteriolar volume density in that region, compared with sham-operated rats. Thus our finding that arteriolar growth was not negated in the middle-aged post-MI rats does not explain the perfusion deficit in the surviving LV myocardium, especially in the septum. More likely is the possibility that vasodilation is restricted in the remaining noninfarcted myocardium of post-MI hearts.

DNA synthesis during adaptive arteriolar growth. Our study is the first to address the issue of cell proliferation in precapillary resistance vessels, i.e., coronary arterioles, after MI in two distinct LV regions, i.e., the noninfarcted free wall and the septum. The increase in DNA synthesis in SM and/or endothelial cells of coronary arterioles 7 and 14 days after MI is consistent with the data of others (14, 19) on capillary endothelial cell DNA synthesis. Moreover, in the LVFW, as well as in the septum, the temporal dynamics of DNA synthesis were comparable in arteriolar SM and endothelial cells. The probability that during post-MI cardiac remodeling wall stress in the LVFW becomes higher earlier than that in the septum (3) may explain why arteriolar cell DNA synthesis in the septum was delayed by nearly a week.
Although the design of our current study did not allow us to speculate about the exact mechanisms that govern the arteriolar growth in noninfarcted LV myocardium of post-MI heart, two triggering factors are most likely. Because our group’s recent study (20) showed that mechanical stretch of rat microvascular endothelial cells in vitro induced upregulation of key angiogenic receptors and, therefore, increased their sensitivity to angiogenic growth factors, we suggest that the mechanical stretch imposed on the vessels during post-MI cardiac remodeling may be one possible factor underlying the stimulus for arteriolar growth. In support of this hypothesis is the finding that diastolic wall stress, and hence the extravascular stretch, was increased in both the LVFW and septum of post-MI rats (3). Another triggering factor may be the elevated demand in O2 supply in overloaded cardiac myocytes. Because a greater diastolic wall stress in the LVFW, compared with that in the septum of post-MI hearts (3), induces a greater compensatory hypertrophy (1, 7, 8, 21), the cardiac myocytes in the LVFW required a greater O2 supply than those in the septum, where no concentric myocyte enlargement was detected. The dual effect of the greater vascular stretch and compensatory myocyte growth may explain why the arteriolar growth in the LVFW was greater than that in the septum.

Angiogenic growth factor/receptor upregulation during adaptive arteriolar growth. Although previous studies (10, 11, 15, 16, 21) have confirmed the involvement of the VEGF/Flt-1/Ang-1 and Ang-1/Ang-2/Tie-2 angiogenic regulatory systems in MI-induced growth of coronary vasculature in the surviving LV myocardium, our study is the first to compare the temporal upregulation of these angiogenic regulatory systems during adaptive arteriolar growth in the noninfarcted free wall and the septum. Most of the previous studies (10, 11, 15, 16, 21) focused on the peri-infarct zone, i.e., tissue adjacent to the edge of infarct. However, this region is unique because it is closely related to scar-formation process and undergoes intensive angiogenesis as early as 48 h after MI (2). Therefore, upregulation of angiogenic growth factors and their receptors in such areas are principally triggered by the reparative process itself.

In contrast to previous studies (15, 16), our current findings documented increased in the levels of Ang-2 and Tie-2 protein expressions on days 7 and 14 after MI, especially in the LVFW. Most importantly, we discovered that the rise in Ang-2 and Tie-2 proteins correlates temporally with activation in arteriolar DNA synthesis. This finding is strengthened by the fact that the peak growth in the arteriolar bed of the septum correlated with the onset of Ang-2 protein upregulation, despite the fact that it occurred a week later than that in the LVFW. At the same time, we found no significant activation in VEGF/Flt-1/Ang-1 protein expression in either of noninfarcted LV regions during these time points. These data are in accordance with earlier findings by Li and colleagues (11), who documented that on day 7 after MI, the levels of VEGF, Flt-1, and Flk-1 mRNA production in noninfarcted LV myocardium were only slightly higher than those in sham-operated rats.

Taken together, our data demonstrate that 1) the overall dynamics of compensatory arteriolar growth, indicated by arteriolar DNA synthesis, was similar between two LV regions, despite some delay in the septum; 2) the Ang-2/Tie-2 angiogenic system is a key player during the compensatory growth of arterioles in the nonischemic myocardium of post-MI hearts; and 3) the perfusion deficit detected in both surviving LV regions cannot be accounted for by inadequate adaptive growth of arterioles.

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