Salvage of Infarcted Myocardium by Angiogenic Action of Basic Fibroblast Growth Factor

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Coronary collateral vessels reduce damage to ischemic myocardium after coronary obstruction. Factors that stimulate collateral formation are expected to have ameliorating effects on myocardial infarction. In a canine experimental myocardial infarct model, intracoronary injection of basic fibroblast growth factor (bFGF) improved cardiac systolic function and reduced infarct size. Treatment with bFGF increased the number of arterioles and capillaries in the infarct. Thus, the angiogenic action of bFGF might lead to a reduction in infarct size. The application of bFGF might bring about a therapeutic modality for the salvage of infarcted myocardium.

Coronary collateral vessels protect myocardial tissues from ischemia during coronary artery stenosis (*1, 2*). An increase in the number of collateral vessels might occur as a result of the enlargement of existing coronary arterial anastomoses or as a result of the creation of new arteries (*3, 4*). Physical factors, such as coronary arterial pressure and flow, influence the growth of existing vessels (*4, 5*). However, other factors that affect the growth of vascular endothelial cells or smooth muscle cells might also influence the creation of vessels. During coronary artery constriction, DNA synthesis and mitosis occur in endothelial, medial, and adventitial cells of collateral vessels and myocardial mesenchymal cells, and these processes may be induced by growth-stimulating factors (*6*). An angiogenic factor of small molecular size is present in tissue from human myocardial infarcts (*7*), and normal myocardium contains heparin-binding endothelial cell mitogens (*8*). In addition, high concentrations of fibroblast growth factors are present in myocardial tissues (*9*).

Fibroblast growth factor (FGF) is a single-chain peptide with a molecular size of 16 kD that induces the growth of fibroblasts (BALB/c 3T3). The most common types of FGF are acidic FGF (aFGF) with a pI of 5 to 7 and bFGF with a pI of 9.6. FGFs are also referred to as heparin-binding growth factors (HBGFs) because of their affinity for heparin. The aFGF exists mainly in brain and retina, whereas bFGF is found in more places, appearing in blood vessels and macrophages as well as in brain. Fibroblast growth factors act as regulatory proteins that induce the proliferation of a variety of cells of epithelial, mesenchymal, and neural origins and that promote the growth and regeneration of organs and tissues in vivo (*10*). Fibroblast growth factor also functions as an angiogenic factor both in vivo and in vitro (*11, 12*). Both FGFs have been reported in myocardial tissues (*9*); however, it is unclear which form is predominant.

Using an enzyme-linked immunosorbent assay (ELISA), we found that canine myocardial infarct tissue contains more bFGF than noninfarct tissue (*13*). Therefore, we tested the effect of an intracoronary injection of bFGF on coronary vasculature and myocardial infarct in dogs subjected to experimental myocardial infarction. Mongrel dogs with masses from 9 to 15 kg were anesthetized with intravenous pentobarbital sodium (25 to 30 mg per kilogram of body mass), intubated, and ventilated with room air. Guiding catheters (9F) were inserted into the ascending aorta and the ostium of the left coronary artery through the right carotid artery. Lidocaine (20 mg) was given intravenously before coronary occlusion and infused (0.8 mg/min) continuously thereafter. Before coronary artery occlusion, coronary angiography (CAG) and left ventriculography (LVG) were performed. Aortic blood pressure and left ventricular pressure were also monitored through the catheter. The acute myocardial infarct was made by the injection of an artificial thrombus into a segment of the coronary artery that had been made stenotic by laser ablation (*14*). The hot-tip probe (1.5 mm in diameter) of an ar laser (Trimedyne, Santa Ana, California) was inserted into the middle portion of the left ascending coronary artery (LAD), and the intima of the coronary artery was ablated (3.5 W for 3 s). We then performed CAG to ascertain that coronary artery stenosis (from 50 to 90% of control) had occurred.

The artificial thrombus was made of a combination of arterial blood (2 ml), fibrinogen (20 mg), and thrombin (300 units). We placed the thrombus (2 × 2 × 8 mm) in the stenotic portion of the LAD with a 6F catheter in order to occlude the coronary artery. Thirty minutes and 6 hours after coronary artery occlusion, CAG and LVG were performed, and human recombinant bFGF (10 μg in 10 ml of saline) (Earth Chemical, Akou, Japan) was infused at a constant speed for 1 min into the left circumflex coronary artery (LCX). After checking the animal's status, we removed the catheters and sutured the incised portion of the neck. Antibiotics (penicillin and kanamycin) were injected intramuscularly. All dogs were cared for in accordance with institutional guidelines.

The dogs were again anesthetized 1 week later, and guiding catheters (9F) were inserted into the left carotid artery. After CAG and LVG were performed, we injected Evan's blue dye into the coronary artery to stain the noninfarct area, and we then injected paraformaldehyde (4%) into the coronary artery to fix the coronary vasculature in vivo. After cardiac arrest, the hearts were excised and cut cross-sectionally into plates 5 mm thick. The infarct was identified by obvious necrosis and fibrosis in the nonstained area, and it was histologically examined. Control animals were operated on as well; an infarct was made in the same manner, and the vehicle (10 ml of saline) was injected into the coronary arteries. We used the paired or unpaired *t* test to assess the statistical significance of differences between variables. There was no alteration associated with bFGF injection in blood pressure, left ventricular pressure, or heart rate in dogs throughout the experiment (*13*). Furthermore, no obvious adverse effect, such as an anaphylactic reaction, was elicited by the injection of human bFGF, and we did not use any drugs to prevent such reactions.

To determine the effect of bFGF on left ventricular function, we calculated the left ventricular ejection fraction (LVEF) from LVG (*15*). In the control animals, LVEF decreased significantly during the first week after occlusion (before occlusion, 0.57 ± 0.05; 1 week after, 0.24 ± 0.05; $P < 0.05$). In bFGF-treated animals at 1 week after occlusion, LVEF was significantly recovered from the depressed

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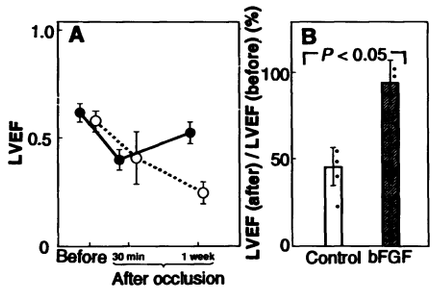

Fig. 1. Effects of bFGF treatment on LVEF before and after occlusion of the LAD. Time course of changes in LVEF from before occlusion to 1 week after occlusion (**A**) and the ratio of LVEF at 1 week after occlusion to LVEF before occlusion (**B**). We performed LVG with contrast medium by inserting a catheter into the left ventricle through the carotid artery before occlusion of the LAD, 30 min after occlusion, and 1 week after occlusion. A right anterior oblique view of LVG was traced in the systolic and diastolic phases of contraction, and LVEF was calculated (*15*) with a computer analysis system (Angiographic Ventricular Dynamics Systems, Version 2.1, Siemens, Solna, Sweden). The bFGF (10 μg) dissolved in 10 ml of saline or vehicle was injected into the LCX at 30 min after occlusion and again 6 hours after occlusion. Closed circles in (A) indicate the mean LVEF ± SEM of five FGF-treated dogs, and open circles indicate that of five control dogs. Bar heights in (B) are the means ± SEM (n = 5 for each treatment); dots indicate the raw data.

value at 30 min after occlusion (30 min after, 0.40 ± 0.05; 1 week after, 0.52 ± 0.05; $P < 0.05$) (Fig. 1A). The ratio of LVEF at 1 week after occlusion to that before occlusion was significantly higher in the FGF-treated group than in the control group (94 ± 12 and 46 ± 11%, respectively; $P < 0.05$) (Fig. 1B). Although there was no difference in left ventricular wall weight in hearts excised 1 week after occlusion, the ratio of infarct weight to left ventricular wall weight was significantly smaller in the bFGF-treated group than in the control group (5.5 ± 1.4 and 19.9 ± 2.4%, respectively; $P < 0.01$) (Fig. 2). Therefore, it appears that bFGF improved cardiac function by salvaging ischemic myocardium from necrosis and by reducing infarct size.

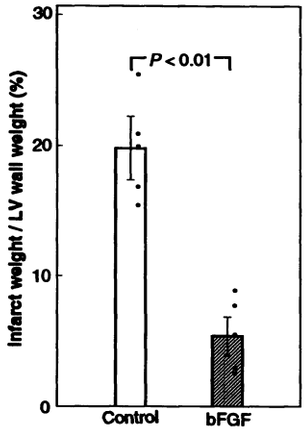

Fig. 2. Effects of bFGF treatment on the size of myocardial infarct at 1 week after LAD occlusion. The contour of the infarct and left ventricle were traced on the surface of cross-sectional preparations, and the ratio of the infarct to total left ventricular wall, including intraventricular septum, was calculated. We calculated the ratio of infarct weight to left ventricular weight by multiplying this ratio by the weight of each cross section. Bar heights are means ± SEM of the ratio of infarct weight to left ventricular wall weight. The dashed bar shows the mean of five FGF-treated dogs, and the open bar that of five control dogs. Dots indicate raw data. LV, left ventricle.

In order to elucidate the mechanisms by which bFGF protects myocardium from necrosis or ischemic injury, we studied the effect of FGF on coronary vasculature. We performed CAG before coronary artery occlusion and 30 min, 6 hours, and 1 week after occlusion. To provide full visualization of small coronary arteries, we took care to inject contrast medium with enough force and in sufficient quantity to selectively make the LCX opaque. At 30 min or 6 hours after occlusion, there was no significant change in angiographs of the LCX in either group of dogs. However, 1 week after occlusion (Fig. 3A), the number of small arteries (~100 μm in diameter) that branched from the distal portion of the LCX in the direction of the infarct was significantly increased in bFGF-treated dogs. In control dogs, the number of these branches detected before occlusion did not change significantly during the first week after occlusion. The ratio of the number of these branches at 1 week after occlusion to that before occlusion was significantly higher in the bFGF-treated group than in the control group (170.2 ± 20.4 and 108.9 ± 5.8%, respectively; $P < 0.05$) (Fig. 3B).

Histological study of the excised hearts confirmed this increase in vascular number. Capillaries and arterioles were counted in preparations stained with hematoxylin-eosin and elastica van Gieson. There were more capillaries and arterioles around the LCX in the bFGF-treated group than in the control group (Fig. 4). In infarct that was bordered by a noninfarct area in the direction of the LCX, there was a significant increase in the number of capillaries and arterioles (10 to 50 μm in diameter) per unit area (500 μm by 500 μm) in the bFGF-treated group as compared with the control group (capillaries, 99.0 ± 3.4 and 66.8 ± 6.3, respectively, $P < 0.01$; arterioles, 14.3 ± 0.6 and 5.8 ± 0.5, respectively, $P < 0.01$) (Figs. 5 and 6).

These effects of bFGF on the number of coronary capillaries and arterioles are probably due to its potent angiogenic action (*12*). Whether the capillaries and arterioles that appear in canine cardiac tissue treated with FGF function as well as existing vessels is not clear; however, newly created arterioles and capillaries can function as perfusing vessels (*16*). The beneficial function of coronary collateral vessels in salvaging the myocardium from ischemia has been discussed in experimental animal models of coronary stenosis or obstruction (*4*) and in clinical studies of ischemic heart disease (*2, 4, 17–20*).

The active proliferation of vascular endothelial cells or smooth muscle cells in the area of developing collaterals might indi-

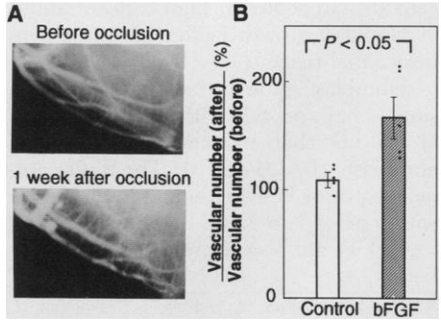

Fig. 3. Effects of FGF treatment on the number of small coronary arteries made opaque by CAG. A representative example of selective angiography of the LCX from a bFGF-treated dog before occlusion and 1 week after occlusion (**A**) and the ratio of the number of opaque small coronary arteries branching from the distal portion of the LCX at 1 week after occlusion to that before occlusion (**B**) are shown. CAG was performed before LAD occlusion, 30 min after occlusion, 6 hours after occlusion, and 1 week after occlusion. We selectively made the LCX opaque with Iopamidole in sufficient quantity to provide full visualization of small coronary arteries branching from the LCX (more than 100 μm in diameter). Numbers of visible branches of the LCX were counted by at least two experienced cardiologists, who were unaware of the group identity of the angiographic films. Interobserver variation was <5%. After 1 week, there was an increase in small coronary arteries branching from the LCX in the FGF-treated dogs, but not in the control dogs. (A) An example of selective angiography of the LCX from an FGF-treated dog is shown. There is an increase in small coronary arteries branching from the LCX at 1 week after occlusion of the LAD (lower panel) compared with before occlusion (upper panel). (B) The change in the number of these arteries was expressed as the ratio of the number of arteries at 1 week after occlusion to that before occlusion. Bar heights are means ± SEM of this ratio (n = 5); dots indicate raw data.

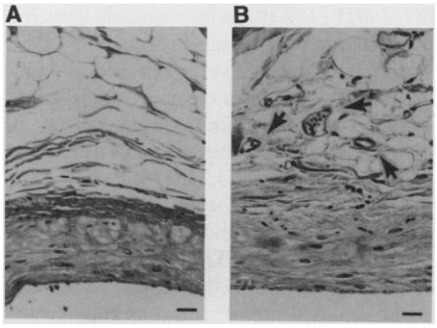

Fig. 4. Micrographs of heart tissues around the LCX (mid portion) in a control dog (**A**) and in a bFGF-treated dog (**B**). Heart tissues were fixed with formaldehyde (10%) and stained with hematoxylin-eosin. Scale bars at the bottom are 30 μm. Arrows in (B) indicate increased capillaries and arterioles.

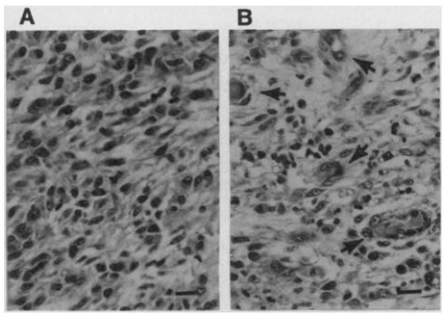

Fig. 5. Micrographs of the myocardial infarct that was bordered in the direction of the LCX by a noninfarct area in a control dog (**A**) and in a bFGF-treated dog (**B**). Tissues were stained as described in Fig. 4. Bars at the bottom indicate 20 μm. Arrows in (B) indicate increased arterioles (from 10 to 50 μm in diameter).

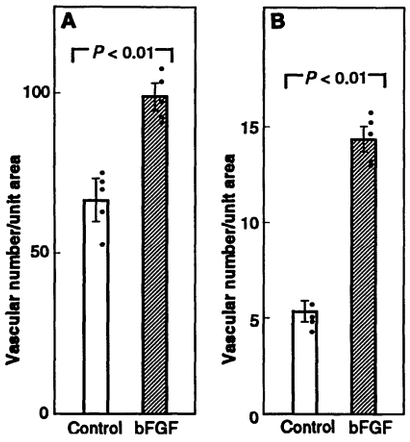

Fig. 6. Effects of bFGF on the number of capillaries (**A**) and arterioles (**B**) in infarct. At 1 week after occlusion of the LAD, capillaries and arterioles were counted in the preparations stained with elastica van Gieson by at least two experienced pathologists, who were unaware of the group identity of the slides. Interobserver variation was <5%. Five fields (5 mm by 5 mm) were randomly chosen from the myocardial infarct that was bordered by a noninfarct area in the direction of the LCX. Researchers assessed the density of arteries and capillaries in each 5 mm by 5 mm field by counting the mean number of vessels in five randomly chosen unit areas (500 μm by 500 μm) using a section ocular micrometer (Olympus, Tokyo, Japan) at ×400 magnification (*25*). The total number of vessels in 25 unit areas (five fields with five unit areas in each field) were counted. Bar heights are means ± SEM (n = 5); dots indicate the raw data.

cate that growth factors influence the creation of collateral vessels (*21*). Our results suggest that one of these factors might be bFGF. Heparin, which is known to release endogenous bFGF from extracellular matrix, or heparan sulfate (*22*) stimulated collateral formation (*23*). In addition, protamine inhibits capillary growth in the coronary vasculature by its antagonistic action against heparan (*24, 25*). According to these reports, bFGF might function as an important physiological regulator of coronary collateral formation.

We used immunohistochemical staining to investigate the distribution of administered bFGF. The sites most stained were the vascular endothelium of the LCX, through which bFGF was injected, and infarct that was bordered by a noninfarct area in the direction of the LCX (*13*). The bFGF adheres to extracellular matrix, such as heparan sulfate proteoglycans, and is gradually released from the extracellular matrix by heparinase and proteolytic enzymes (*22*). Therefore, the deposition of bFGF might have resulted from the retention of bFGF by the extracellular matrix in these areas. Through these mechanisms, bFGF injected in bolus form and retained in the cardiac tissue of the myocardial infarction model might be released gradually and cause the creation of vessels seen 1 week later. In our canine myocardial infarct model, vessel formation occurred within 1 week after the administration of bFGF. This comparatively rapid process of vessel formation is in accordance with results indicating that coronary collateral development occurred in dogs within 3 to 11 days after coronary occlusion (*26, 27*).

The bFGF may promote the proliferation of vascular smooth muscle cells and fibroblasts. The proliferative responses of vascular smooth muscle cells after endothelial injury by angioplasty or experimental denudation are mediated by endogenous or exogenous bFGF, or both (*28, 29*). In contrast to these results, intimal thickening owing to smooth muscle cell proliferation was not observed at 1 week after treatment with bFGF in the present study. The inconsistency between our results and those of others (*28, 29*) might be due to differences in the doses of FGF [two 10-μg doses per dog weighing 9 to 15 kg; and 120 μg per rat weighing 280 to 340 g (*29*)], the method of administration [two bolus injections through the LCX and bolus injection followed by continuous infusion through the aorta (*29*)], and the status of the vascular endothelial cells [nondenuded endothelium and denuded endothelium (*29*)]. We administered a lower dose of bFGF through the LCX and were careful to minimize endothelial cell injury. Under these conditions, bFGF did not cause intimal thickening through the proliferation of smooth muscle cells. Therefore, bFGF might be therapeutic in cases of infarcted myocardium.

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