Effect of Basic Fibroblast Growth Factor on Left Ventricular Geometry in Rats Subjected to Coronary Occlusion and Reperfusion

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Abstract

Background: Previous studies have demonstrated myocardial salvage by basic fibroblast growth factor administration following chronic myocardial ischemia or acute myocardial infarction.

Objectives: To study the effect of bFGF on left ventricular morphometry following coronary occlusion and reperfusion episode in rats.

Methods: bFGF (0.5 mg) or placebo was continuously administered for a period of one week using an implanted osmotic pump. Animals were sacrificed 6 weeks after surgery and myocardial cross-sections were stained with Masson-trichrome and with anti-proliferating cell nuclear antigen antibody.

Results: LV area, LV cavity diameter, LV cavity/wall thickness ratio, and injury size were unchanged compared with control animals. Proliferating endothelial cells were significantly more abundant in injured compared with normal myocardium, but with no differences between animals treated or not treated with bFGF.

Conclusions: One week of systemic bFGF administration following coronary occlusion and reperfusion had no additional effect on LV geometry or cellular proliferation in rats.

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Successful coronary reperfusion by either pharmacologic (thrombolysis) or mechanical (coronary angioplasty) means preserves left ventricular function and reduces the morphologic changes associated with post-infarction left ventricular remodeling [1]. It was previously shown that ischemia stimulates the expression of different mitogenic growth factors such as fibroblast growth factor [2], vascular endothelial growth factor [3] and transforming growth factor [4], which can induce capillary growth and angiogenesis [4–6]. Moreover, exogenous administration of fibroblast growth factor or vascular endothelial growth factor to different animal species with induced chronic myocardial ischemia or acute infarction has been shown to increase myocardial perfusion and preserve left ventricular function [7,8]. However, since many patients benefit from successful reperfusion following acute infarction, the use of angiogenic therapy under such condition warrants examination.

Recently, Padua et al. [9] showed that bFGF increased cardiac resistance to ischemic injury and improved functional recovery in an isolated rat heart model of ischemia and reperfusion. The present study was undertaken to examine the effectiveness of angiogenic treatment by bFGF in an *in vivo* rat model of coronary occlusion following reperfusion.

bFGF = basic fibroblast growth factor
LV = left ventricular

Materials and Methods

The protocol was approved by the Institutional Committee, and the experiments were conducted in accordance with the "Guide for the Care and Use of Laboratory Animals" (National Academy Press, Washington DC, 1996).

Experimental protocol

Thirty male Sprague-Dawley rats weighing 344 ± 14 g were divided into two groups of 15 animals each. Group 1 was subjected to coronary occlusion for 45 minutes, followed by reperfusion, and treated with bFGF (bFGF-treated group). Group 2 was exposed to coronary occlusion, followed by reperfusion, and treated with rat albumin (bFGF-untreated group). Five animals did not undergo operation (no ischemia) and served as the control group. The bFGF in a dose of 0.5 mg/week, or rat

albumin 1 mg/week, was continuously administered into the peritoneum, using an implanted osmotic pump as we have described previously [10]. The rats were sacrificed 6 weeks after surgery, and the hearts were perfused-fixed at physiologic pressure for subsequent morphometric analysis.

Surgical preparation

Following anesthesia with diethyl ether, a left thoracotomy was performed, the heart was exteriorated by light pressure on the thorax, and a branch of the left coronary system was ligated with a 4/0 silk suture, leaving the distal end of the suture outside the chest. The heart was repositioned in the thorax and the chest was immediately closed in layers [11]. Forty-five minutes after ligation, the animals were re-anesthetized and reperfusion was performed by gently pulling the distal end of the suture.

Alzet osmotic pumps (ALZA Corporation, USA, pump model 2001) were loaded with either 0.5 mg of human recombinant bFGF (Amgen Inc, Boulder, CO, USA), or with 1 mg of rat albumin alone. Pumps were implanted surgically in the peritoneal cavity immediately after reperfusion, and the incision site was closed by sutures. Since the osmotic pumps start to deliver their contents only a few hours after implantation, a dose of 70 μg bFGF or rat albumin was injected intraperitoneally immediately after implantation of the pump. The investigators were blinded to the contents of the pumps and the injections.

Perfusion fixation

Six weeks after surgery all animals were anesthetized, the abdomen was opened, and a polyethylene catheter was introduced into the descending aorta. Heparin (7,500 U) followed by KCl solution (15%) was administered to kill the animals. The chest was opened and the right atrial appendage was removed. The heart was perfused retrogradely with 10% phosphate-buffered formalin at a constant pressure (60 mmHg) for 30 min. After hardening, the hearts were excised and immersed in 10% buffered formalin solution until analysis.

Histology and morphometry

The apical region of all hearts was removed 5 mm from the apex. Cross-sections (5 μm thick) were cut from the remaining myocardium, at 5 mm and 6 mm from the apex, three sections at each level, and stained with Masson-trichrome (differentiating between viable tissue, which was stained violet – and scar tissue, which was stained green), hematoxylin and eosin, and immunohistologic staining with antibodies against PCNA [12].

Masson-trichrome stained slides were analyzed by a computerized colored image analyzer (Supercue-3, Galai, Migdal HaEmek, Israel) for the following parameters: left ventricular area (mm^2), LV cavity diameter (mm), non-ischemic and injured areas (mm^2, %), and regional thickness of injured and non-ischemic wall (mm). The LV cavity area/wall thickness ratio was calculated. The parameters reflecting the coronary occlusion-induced injury were not analyzed in control animals. All measurements were performed on two slices from each heart, from 5 and 6 mm from the apex, and at a magnification of x10. The results are expressed as an average of the two cross-sections.

Assessment of cellular proliferation

Cross-sections were stained with mouse anti-proliferating cell nuclear antigen antibody according to the method of Casasco et al. [12]. Slides were analyzed by an investigator blinded to the treatment, using an Olympus light microscope (model BH2) at a magnification of x400. In each slide, areas (0.11 mm^2) representing normal, border or coronary occlusion-induced injury were analyzed. PCNA-stained endothelial cells (any brown-stained endothelial cells around vascular lumen) and non-endothelial fibroblast-like cells (all other positively stained cells within each slide) were counted [13], and the results are expressed as cells/mm^2.

Data analysis

The mean and standard deviation of each variable was calculated. A General Linear Models Procedure was performed in ANOVA using the software [14] to compare between the three experimental groups. $P \leqslant 0.05$ was considered significant. The data are expressed as mean $\pm$SD.

Results

Body weight

There were no differences in body weight before and after the experiment between bFGF-treated and bFGF-untreated groups (357 ± 20 vs. 353 ± 16 g before and 496 ± 17 vs. 511 ± 14 g after the experiment).

bFGF effect on LV morphometry

LV cavity diameter [Figure 1A] was larger in both bFGF-untreated and bFGF-treated groups compared with the control group (5.5 ± 1.6, 6.0 ± 1.1, and 4.4 ± 0.8 mm, respectively, $P = 0.05$), with no difference between bFGF-treated and untreated groups. The LV area [Figure 1B] was similar in all groups: 43.0 ± 1.5, 44.3 ± 6.7, and 44.4 ± 7 mm^2 for control, bFGF-untreated, and bFGF-treated groups, respectively (P=NS).The LV cavity/wall thickness ratio was higher in both bFGF-untreated and bFGF-treated rats than in the control group [Figure 1C], but with no statistical differences between the two groups (3.9 ± 1.8 vs. 4.4 ± 1.4 for bFGF-untreated and bFGF-treated groups, respectively, P=0.2).

The ischemic area was detected by fibrotic fibers forming scar tissue within the injured myocardial wall. The size of the injured area was small, with no difference between bFGF-untreated and bFGF-treated animals (4.4 ± 2.5 and 5.1 ± 3.4%, respectively, P=0.3) [Table 1]. The infarcted wall was significantly thicker in bFGF-untreated compared with bFGF-treated rats (1.7 ± 0.4 and 1.5 ± 0.2 mm, respectively, $P = 0.04$), while the thickness of non-injured walls [Table 1] was not different among groups.

PCNA = proliferating cell nuclear antigen

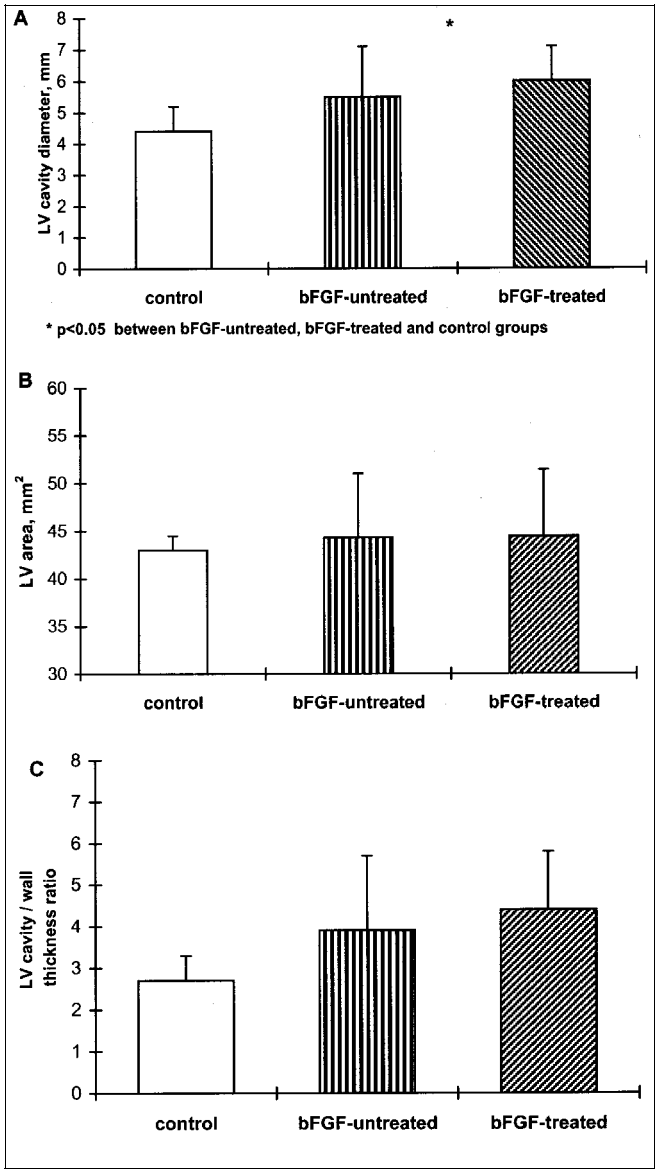

Figure 1. Differences in LV cavity diameter (mm) **[A]**, LV area (mm^2) **[B]**, and LV cavity/non-myocardial infarction wall thickness ratio **[C]** in bFGF-treated, bFGF-untreated, and control groups.

* denotes a significant difference between bFGF-treated and bFGF-untreated groups versus the control group.

Table 1. Changes in infarct size and wall thickness of the infarcted and non-infarcted myocardium in bFGF-treated and bFGF-untreated groups

Group	Infarct size (%)	Wall thickness of infarct area (mm)	Wall thickness of non-infarct area (mm)
bFGF-untreated	4.4 ± 2.5	1.7 ± 0.4*	1.5 ± 0.2
bFGF-treated	5.1 ± 3.4	1.5 ± 0.2	1.4 ± 0.2
Control			1.6 ± 0.1

Changes in infarct size and wall thickness in bFGF-treated and bFGF-untreated animals (data are mean ± SD).

* $P = 0.04$ between groups

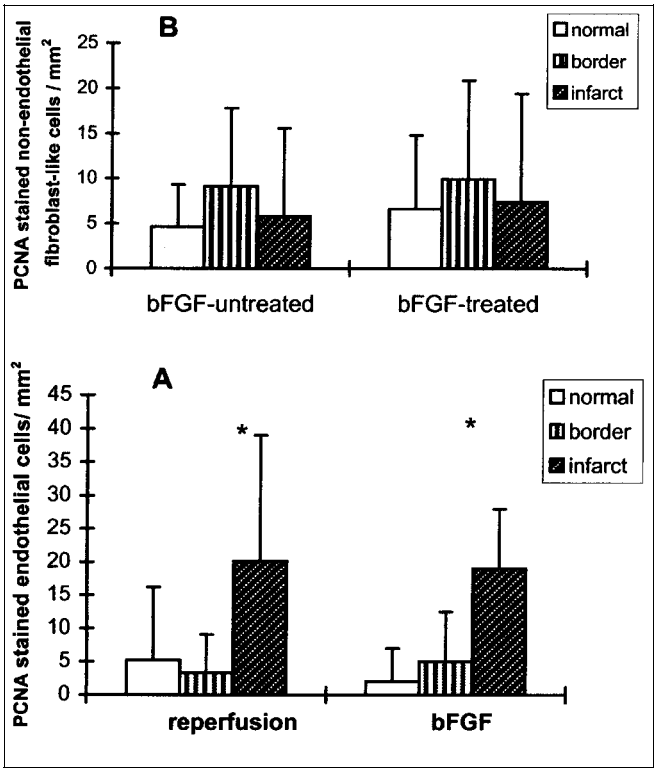

Figure 2. PCNA-stained endothelial cells (per mm^2) **[A]** and PCNA-stained non-endothelial fibroblast-like cells (per mm^2) **[B]** in normal, border and infarcted myocardium in both experimental groups.

* denotes a significant difference between infarct zone, normal, and border zones.

Cellular proliferation

There were more PCNA-stained cells in the injured than in the border or normal area of the LV [Figure 2A]. The number of endothelial cells in the ischemic zone and in the normal zone in the bFGF-untreated animals was $20 \pm 19/mm^2$ and $5 \pm 10/mm^2$, respectively, $P = 0.001$. In the bFGF-treated animals there were 19 ± 9 endothelial cells/mm^2 in the injured and 2 ± 5 endothelial cells/mm^2 in the normal region ($P = 0.001$). However, no differences were found between the bFGF-untreated and bFGF-treated groups. No differences were observed in non-endothelial fibroblast-like cells between all regions and between the two groups [Figure 2B].

Discussion

The aim of the present study was to investigate whether bFGF administration has an additional beneficial effect on reperfusion following coronary occlusion in rats. The results do not demonstrate any supplementary effect of bFGF on either left ventricular geometry or cellular proliferation 6 weeks after a successfully performed reperfusion.

Previous studies in rats have shown that early and late reperfusion following acute ischemia reduces the extent of the infarction and preserves the geometry of the left ventricle [15,16]. Furthermore, administration of bFGF following short

episodes of coronary occlusion reduced the post-ischemic reperfusion injury in rats [17] and reduced infarct size in dogs [18]. In the present study bFGF was administered for 1 week starting immediately after coronary occlusion and reperfusion. We found that reperfusion alone increased LV area, reduced LV chamber size, and increased wall thickness of the non-injured myocardium. These changes were not altered by the administration of bFGF.

In previous studies, bFGF was administered exogenously for 1–6 weeks and was effective in enhancing collateral growth following chronic coronary artery occlusion [19,20]. bFGF administered directly into the coronary bed reduced infarct size, preserved ventricular function, and increased the number of arteriolar capillaries following acute infarction [7]. Intramyocardial infusion of bFGF for 60 minutes prior to 60 min left anterior descending artery occlusion, followed by 120 min reperfusion, significantly decreased infarct area in swine [21]. It was also found that intraperitoneal administration of bFGF induced myocardial hypertrophy following acute infarction in rats [10]. We decided to administer bFGF for one week, based on data showing growth of collaterals within one week after arterial occlusion [5], and on accumulating data demonstrating angiogenic response within this time frame following angiogenic therapies [19,20].

We chose a dose of 0.5 mg/rat/week (0.285 mg/kg/day) based on our previous studies where similar concentrations of bFGF in different animal models induced angiogenesis or myocardial hypertrophy [7,10,19]. For example, in our dog model of chronic myocardial ischemia, 70 μg/kg of bFGF injected into the left atrium was effective in increasing collateral blood flow [19]. In our pig model of micro-embolization infarction, 0.08 μg/kg bFGF was incorporated in affigel beads injected into the coronary arteries, leading to a significant increase in the number of capillaries [7]. In a rat model of acute coronary occlusion, 0.5 mg of bFGF administered intraperitoneally (using alzet osmotic pumps) induced myocardial hypertrophy 6 weeks post-infarction [10].

Horrigan et al. [18] and Cuevas and colleagues [17] injected 20 μg and 10 μg of bFGF/heart to dogs and rats, respectively, and found a protective effect in both cases. In contrast to our study, Horrigan's group [18] infused bFGF intracoronary, 15 min after the LAD occlusion, while the LAD was occluded for 180 min. Compared with Cuevas's study [17], the dose in our study was three to four times smaller (roughly 250 μg/rat in the Cuevas study vs. 70 μg/rat in our study). Thus the relatively low dose of 0.285 mg of bFGF/kg/day administered continuously in our study may not have contributed to the improved LV morphometry following successful reperfusion. In addition, the size of the injured area in these other studies was larger than in ours [17,18]. Possibly, the reperfusion exhausted the "anti-remodeling" reserve of the left ventricle [22], precluding an additional effect of bFGF. Obviously, there may be other unknown factors responsible for the results.

The high number of PCNA-stained endothelial and non-endothelial fibroblast-like cells in the injured area (fourfold higher than normal or border zones) suggests that the healing process of the reperfused myocardium had not yet been completed by the time the animals were sacrificed. It was previously shown that the mitotic index of endothelial cells was high (threefold higher than control) 6 weeks after bFGF administration in a dog model of progressive coronary artery occlusion [23]. In our model, administration of bFGF, a known mitogenic growth factor [24] during the first week after reperfusion had no effect on cellular proliferation in all regions.

Limitations

We based our analysis of left ventricular remodeling on morphometric changes obtained from cross-sections of the perfused-fixed myocardium. While this method was shown previously to correlate well with myocardial function [25], it may underestimate the changes that occur following bFGF administration in myocardium with diminutive injury. Thus, successfully performed reperfusion altered the injury size, such that the last injury size may have been too small to achieve any effect induced by the FGF peptide.

Conclusions

It is conceivable that successful reperfusion alone is effective in reducing the damage induced by coronary occlusion, so that administration of an angiogenic agent in this setting exerts no further beneficial effects. In the present study, one week of continuous intraperitoneal administration of a relatively small dose of bFGF in rats subjected to coronary occlusion and reperfusion episode did not have any discernible effects on left ventricular geometry. Our study does not contradict the effectiveness of bFGF treatment, though it emphasizes the effectiveness and importance of reperfusion following myocardial coronary occlusion.

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LAD = left anterior descending artery

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