

Intrapericardial Delivery of Fibroblast Growth Factor-2 Induces Neovascularization in a Porcine Model of Chronic Myocardial Ischemia

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Accepted for publication October 21, 1999 This paper is available online at <http://www.jpet.org>

ABSTRACT

Therapeutic angiogenesis is a novel approach to the treatment of myocardial ischemia based on the use of proangiogenic growth factors to induce the growth of new blood vessels to supply the myocardium at risk. This study was designed to assess the safety and efficacy of a single intrapericardial injection of basic fibroblast growth factor (FGF-2) in a porcine model of chronic myocardial ischemia. Yorkshire pigs underwent ameroid placement around the left circumflex coronary artery. At 3 weeks, animals were randomized to receive a single intrapericardial injection of either saline ($n = 10$), 3 mg of heparin ($n = 9$), 3 mg of heparin + 30 μ g of FGF-2 ($n = 10$), 200 μ g of FGF-2 ($n = 10$), or 2 mg of FGF-2 ($n = 10$). Coronary angiography, microsphere flow, magnetic resonance functional, and perfusion imaging were performed before and 4 weeks after treatment, at which time histologic analysis was

also performed on 3 animals in each group. In ischemic pigs, FGF-2 treatment resulted in significant increases in left-to-left angiographic collaterals and left circumflex coronary artery blood flow. These benefits were accompanied by improvements in myocardial perfusion and function in the ischemic territory, as well as histologic evidence of increased myocardial vascularity without any adverse effects. Not one of these benefits was seen in saline- or heparin-treated ischemic animals. A single intrapericardial injection of FGF-2 in a porcine model of chronic myocardial ischemia results in functionally significant myocardial angiogenesis, without any adverse outcomes. This mode of FGF-2 administration may prove to be a useful therapeutic strategy for the treatment of patients with ischemic heart disease.

Ischemic heart disease remains the leading cause of morbidity and mortality in the Western hemisphere. The current management of chronic myocardial ischemia includes therapies that reduce myocardial oxygen demand or increase blood supply to compromised territories (coronary artery bypass grafting and percutaneous transluminal coronary angioplasty). Therapeutic myocardial angiogenesis is a novel approach to the treatment of myocardial ischemia based on the use of proangiogenic growth factors to induce the growth and development of new blood vessels to supply the myocardium at risk. Angiogenesis is a complex process involving endothelial and smooth muscle cell proliferation and migration, formation of new capillaries, and extracellular matrix turnover (Ware and Simons, 1997; Laham and Simons, 1999). We and others have shown that various heparin-binding growth fac-

tors, including basic fibroblast growth factor (FGF-2) (Yanagisawa-Miwa et al., 1992; Battler et al., 1993; Harada et al., 1994; Landau et al., 1995; Lazarous et al., 1996), acidic fibroblast growth factor, and vascular endothelial growth factor (VEGF) (Banai et al., 1994; Pearlman et al., 1995; Engler, 1996; Harada et al., 1996; Lazarous et al., 1996) induce angiogenesis in chronic myocardial ischemia. Given the typically long time course of new collateral vessel development, most attempts to stimulate myocardial angiogenesis have used methods of prolonged growth factor delivery, including gene therapy, continuous infusions, repeated injections, or sustained release polymers (Battler et al., 1993; Banai et al., 1994; Unger et al., 1994; Landau et al., 1995; Mesri et al., 1995; Harada et al., 1996; Lazarous et al., 1996; Magovern et al., 1997; Shou et al., 1997). However, some of these options are not feasible or practical in patients with ischemic heart disease, making single dose administration, if effective, a potentially superior strategy in these patients.

The pericardial space may potentially serve as a drug

Received for publication August 31, 1999.

¹ This work was supported in part by National Institutes of Health Grants MO1-RR01032 (to R.J.L.), HL 53793 and HL 56993 (to M.S.), HL 55394 (to J.D.P.), HL 42279 (to F.W.S.), and a Research Award from Chiron Corporation.

ABBREVIATIONS: FGF-2, basic fibroblast growth factor; LAD, left anterior descending coronary artery; LCX, left circumflex coronary artery; VEGF, vascular endothelial growth factor; MRI, magnetic resonance imaging; AU, optical absorbance; EF, ejection fraction.

delivery reservoir that might be used to deliver therapeutic agents to the heart. Chronic intrapericardial FGF-2 delivery in a rabbit model of angiotensin II-induced cardiac hypertrophy resulted in a localized myocardial angiogenic response (Landau et al., 1995). A single intrapericardial injection of FGF-2 with or without heparin resulted in localized angiogenesis and myocardial salvage in a canine model of myocardial infarction (Uchida et al., 1995). Moreover, the concentration of FGF-2 and VEGF in the pericardial fluid of patients with unstable angina has been documented to be higher than that in patients with nonischemic heart disease, suggesting that increases in the levels of proangiogenic growth factors in the pericardial space may reflect an endogenous and, indeed, physiological response to myocardial ischemia and injury (Fujita et al., 1996). Accordingly, the pericardium may serve as a useful reservoir for proangiogenic growth factor administration in patients with coronary disease. This study was designed to assess the safety and efficacy of a single intrapericardial injection of FGF-2 in a porcine model of chronic myocardial ischemia.

Materials and Methods

Chronic Myocardial Ischemia Model. Yorkshire pigs of either sex weighing 15 to 18 kg (5–6 weeks old) were anesthetized with i.m. ketamine (10 mg/kg) and halothane by inhalation. A right popliteal cut-down was performed and a 4 French arterial catheter was inserted for blood sampling and pressure monitoring. Left thoracotomy was performed through the 4th intercostal space. The pericardium was opened, and an ameroid constrictor of 2.5 mm i.d. (matched to the diameter of the artery) was placed around the left circumflex coronary artery (LCX) (Harada et al., 1994, 1996; Unger et al., 1994). The pericardium was closed using 6–0 Prolene suture, (J&J Ethicon, Cincinnati, OH) and the chest was closed. A single dose of i.v. cefazolin (70 mg/kg) was given, and i.m. narcotic analgesics were administered as needed. Animals then were allowed to recover for 3 weeks (time sufficient for ameroid closure) before growth factor delivery. The treatment of animals was based on the National Institutes of Health guidelines, and the protocol was approved by the Institutional Animal Care and Utilization Committee of the Beth Israel Deaconess Medical Center.

Growth Factor Delivery. Three weeks after ameroid placement, animals were anesthetized with i.m. ketamine (10 mg/kg) and isoflurane by inhalation. A right femoral cut-down was performed and an 8 French arterial sheath was inserted for blood sampling, pressure monitoring, and left heart catheterization. Coronary angiography was then performed in multiple views using a 7 French JR4 diagnostic catheter (Cordis, Miami, FL) to confirm LCX occlusion and to assess the extent of collateral circulation in the LCX distribution ("collateral index"). After LCX occlusion was documented, percutaneous subxyphoid pericardial access was undertaken. With the animals in the supine position, the epigastric area was prepped and draped. An epidural introducer needle (Tuohy-17) was advanced gently under fluoroscopic guidance with a continuous positive pressure of 20 to 30 mm Hg. Entry into the pericardial space was confirmed by the injection of 1 ml of diluted contrast. A soft floppy-tipped guidewire was then advanced into the pericardial space and the needle was exchanged for a 4 French infusion catheter.

The animals were then randomized to one of five treatment groups:

1. Control: intrapericardial saline ($n = 10$).
2. Heparin: intrapericardial heparin (3 mg, $n = 9$).
3. FGF-2 30 μ g: intrapericardial FGF-2 (30 μ g) + 3 mg of heparin ($n = 10$).

4. FGF-2 200 μ g: intrapericardial FGF-2 (200 μ g) + 3 mg of heparin ($n = 10$).
5. FGF-2 2 mg: intrapericardial FGF-2 (2 mg) + 3 mg of heparin ($n = 10$).

The infusate was diluted to 10 ml with saline and infused over 5 min with continuous electrocardiographic and pressure monitoring. The catheter was withdrawn, and a set of colored microspheres (blue) was injected into the left atrium to obtain baseline (pretreatment) myocardial blood flow. Finally, a magnetic resonance study was carried out to obtain quantitative measures of global and regional left ventricular function [ejection fraction (EF) and radial wall motion] and assessment of perfusion using previously validated myocardial contrast density mapping (Pearlman et al., 1995). The animals then were allowed to recover for 4 weeks.

Final Study. Four weeks after intrapericardial agent administration, all animals underwent final evaluation. Pigs were anesthetized with i.m. ketamine (10 mg/kg) and isoflurane by inhalation. A left femoral cut-down was performed and an 8 French arterial sheath was inserted for blood sampling, pressure monitoring, and left heart catheterization. Coronary angiography was performed again in multiple views. A second magnetic resonance study was carried out for global and regional left ventricular function and myocardial perfusion (Pearlman et al., 1995). Myocardial blood flow was determined using colored microspheres at rest (yellow) and after maximal coronary vasodilation with i.v. adenosine (white). Animals then were euthanized under anesthesia and the heart was obtained for additional analysis. In addition, a detailed macroscopic and histologic postmortem examination was carried out on three animals in each group.

Angiographic Analysis. Coronary angiography was performed in multiple views (right anterior oblique, anteroposterior, and left anterior oblique views for the left coronary artery; right anterior oblique and left anterior oblique for the right coronary artery). Evaluation of angiographic collateral density was performed by two independent angiographers blinded to treatment assignment. Differences in interpretations were resolved by a third angiographer. The collateral index was assessed for left-to-left and right-to-left collaterals using a 4-point scale (0, no visible collateral vessels; 1, faint filling of side branches of the main epicardial vessel without filling the main vessel; 2, partial filling of the main epicardial vessel; and 3, complete filling of the main vessel) (Rentrop et al., 1985).

Myocardial Blood Flow. Colored microspheres ($15 \pm 0.1 \mu$ m diameter; Triton Technology Inc., San Diego, CA) were used to determine coronary blood flow before treatment initiation (blue) and at the time of final study (yellow and white). For determination of coronary flow at 3 and 7 weeks after ameroid placement, an angiographic JR4 catheter was advanced into the left ventricle and manipulated to engage the left atrium outflow by slow counterclockwise rotation of the catheter; catheter position was verified by contrast injection into the left atrium. In addition, mean left atrial pressure was recorded. A set of microspheres (6×10^6) was diluted in 10 ml of saline and injected into the left atrium over 30 s. Reference blood samples were withdrawn by using a syringe pump at a constant rate of 5 ml/min through the femoral artery. At the time of final study, coronary flow was measured at rest and after maximal vasodilation (achieved with the injection of i.v. adenosine, 1.25 mg/kg). After study completion, the heart was excised and regional myocardial blood flow was determined (Harada et al., 1994, 1996). The heart was excised and a 1-cm midtransverse slice was sectioned and cut into eight segments. The tissue samples and the reference blood samples were digested in an 8 M KOH/2% Tween 80 solution and microspheres were collected using a vacuum filter. Dyes from microspheres were extracted using dimethyl formamide. Samples were then analyzed in a spectrophotometer (HP 8452 A; Hewlett Packard, Palo Alto, CA). Regional blood flow was calculated from optical absorbance (AU) measurements corrected by tissue weight as fol-

lows:

Flow to sample

$$= \frac{(\text{AU per sample})(\text{reference withdrawal rate})/\text{wt.}}{(\text{AU per reference sample})} \text{ ml/min/g}$$

MRI. MRI was performed on all animals at the time of treatment initiation and at the time of final study. MRI was carried out in the body coil of a 1.5 Tesla whole body Siemens Vision system (Iselin, NJ) as previously described (Pearlman et al., 1995). The following measurements were performed:

- Determination of resting left ventricular EF (%).
- Analysis of regional wall motion using percentage of wall thickening.
- Determination of the extent of coronary perfusion in the LCX collateral-dependent territory compared with normal myocardium by measuring gadodiamide-enhanced signal intensity in different parts of the left ventricular wall and generating a space-time map of myocardial perfusion (Pearlman et al., 1995). The space-time maps allow the measurement of the extent of the ischemic zone.

Histopathologic Analysis and Toxicology. Complete autopsies were performed on 15 animals (3 animals in each group). Tissues obtained from the liver, lung, kidney, spleen, eye, bone marrow, and stomach were formalin-fixed and paraffin-embedded. Sections (5 μm) were obtained from all tissue samples, stained with hematoxylin/eosin, and examined microscopically. In addition, tissue samples were obtained from pericardium, epicardial coronary artery, and myocardium in the left anterior descending coronary artery (LAD) distribution (normal) and LCX distribution (ischemic). Sections were stained with hematoxylin/eosin as well as by the Verhoeff-Van Gieson method for collagen and elastin. Complete serum chemistry and hematology studies were performed at 3 and 7 weeks in all animals.

Statistical Analysis. Data are expressed as means \pm S.D. Continuous variables are compared by Student's *t* test and ANOVA (Bonferroni-corrected for multiple-group comparisons), whereas categorical variables were compared by χ^2 analysis. Nonparametric variables (collateral index) were compared using the Wilcoxon rank-sum test. All reported *P* values were two-tailed, and a *P* value $\leq .05$ was considered statistically significant. Linear regression was used to detect a dose-dependent effect.

Results

A total of 56 animals survived ameroid placement around the LCX coronary artery with resultant total LCX occlusion at 3 weeks. Seven animals died after being randomized to a treatment group. Six of these seven animals died within 72 h of intrapericardial agent delivery. Of the seven animals deaths, two animals died of hypoxemia (one control animal and one FGF-2 30 μg animal) due to failure of mechanical ventilation before growth factor delivery, four animals died during MRI (three animals died before growth factor delivery

and one after pericardial access and delivery, with two animals randomized to the 200 μg FGF-2 group and two animals in the control group), and one animal died of unknown cause 26 days after growth factor delivery (heparin group). The remaining 49 animals were randomized to each of five treatment groups with 10 animals in each of the FGF-2 and saline control groups and 9 animals in the heparin group. There were no significant hemodynamic effects of intrapericardial FGF-2 administration at any dose (Table 1); no changes in blood pressure, heart rate, or left atrial pressure were observed with drug administration.

Coronary Angiography

Baseline right and left coronary angiography was available on all 49 animals and final angiography was available on 47 animals. Left-to-left collaterals and right-to-left collaterals were measured (collateral index). The extent of left-to-left collaterals pre- (3 weeks after ameroid placement) and post-treatment (7 weeks after ameroid placement) in all groups is shown in Fig. 1, which shows a significant improvement over baseline in the collateral index of all three FGF-2 treatment groups (30 μg , 200 μg , and 2 mg) with no significant improvement noted in control or heparin-treated animals. Only animals in the FGF-2 2 mg group displayed a trend toward improvement in right-to-left collateral index (collateral index increased by 0.67 ± 0.87 , *P* = .06).

Myocardial Blood Flow

To evaluate further the angiogenic potential of intrapericardial FGF-2 in chronic myocardial ischemia, regional myocardial blood flow was measured at different time points using colored microspheres. Three weeks after implantation of ameroid occluders, at the time of intrapericardial drug delivery, resting myocardial blood flow in the LCX territory was similar in all treatment groups [Fig. 2A, baseline coronary flow (ml/min/g): 1.00 ± 0.31 in controls and 0.97 ± 0.23 in heparin-treated animals versus 0.92 ± 0.08 in the 30 μg FGF-2 group, 0.99 ± 0.15 in the 200 μg FGF-2 group, and 1.10 ± 0.14 in the 2 mg FGF-2 group, *P* = .94] and was significantly lower than flow in the LAD territory (LCX flow: 1.00 ± 0.35 ml/min/g versus LAD flow: 1.43 ± 0.43 ml/min/g, *P* < .0001). Four weeks after intrapericardial drug delivery, LCX flow was significantly higher in FGF-2-treated animals than in controls and heparin-treated animals (Fig. 2, ANOVA, *P* = .002). At the time of the final study, coronary flow (ml/min/g) was 1.05 ± 0.21 in controls (*P* = .7 compared with baseline) and 1.09 ± 0.13 in the heparin group (*P* = .19 compared with baseline and *P* = .6 compared with controls) versus 1.31 ± 0.12 in the 30 μg FGF-2 group (*P* = .0001 compared with baseline and *P* = .004 compared with con-

TABLE 1
Hemodynamic effects of FGF-2

Condition	Mean Arterial Pressure			Heart Rate			
	Pre-Tx	Post-Tx	Final	Pre-Tx	Post-Tx	Final	
		<i>mm Hg</i>				<i>beats/min</i>	
Control	87 \pm 13	88 \pm 4	81 \pm 14	104 \pm 14	103 \pm 22	92 \pm 19	
Heparin	87 \pm 8	80 \pm 13	80 \pm 16	100 \pm 6	104 \pm 17	85 \pm 16	
FGF-2 30 μg	82 \pm 7	80 \pm 13	89 \pm 16	98 \pm 11	105 \pm 16	95 \pm 21	
FGF-2 200 μg	88 \pm 8	85 \pm 9	81 \pm 10	111 \pm 12	102 \pm 12	100 \pm 23	
FGF-2 2 mg	85 \pm 9	86 \pm 15	79 \pm 9	105 \pm 20	98 \pm 18	87 \pm 14	

Final, at the time of the final study (7 weeks); Post-Tx, post-treatment administration (3 weeks); Pre-Tx, pre-treatment administration (3 weeks).

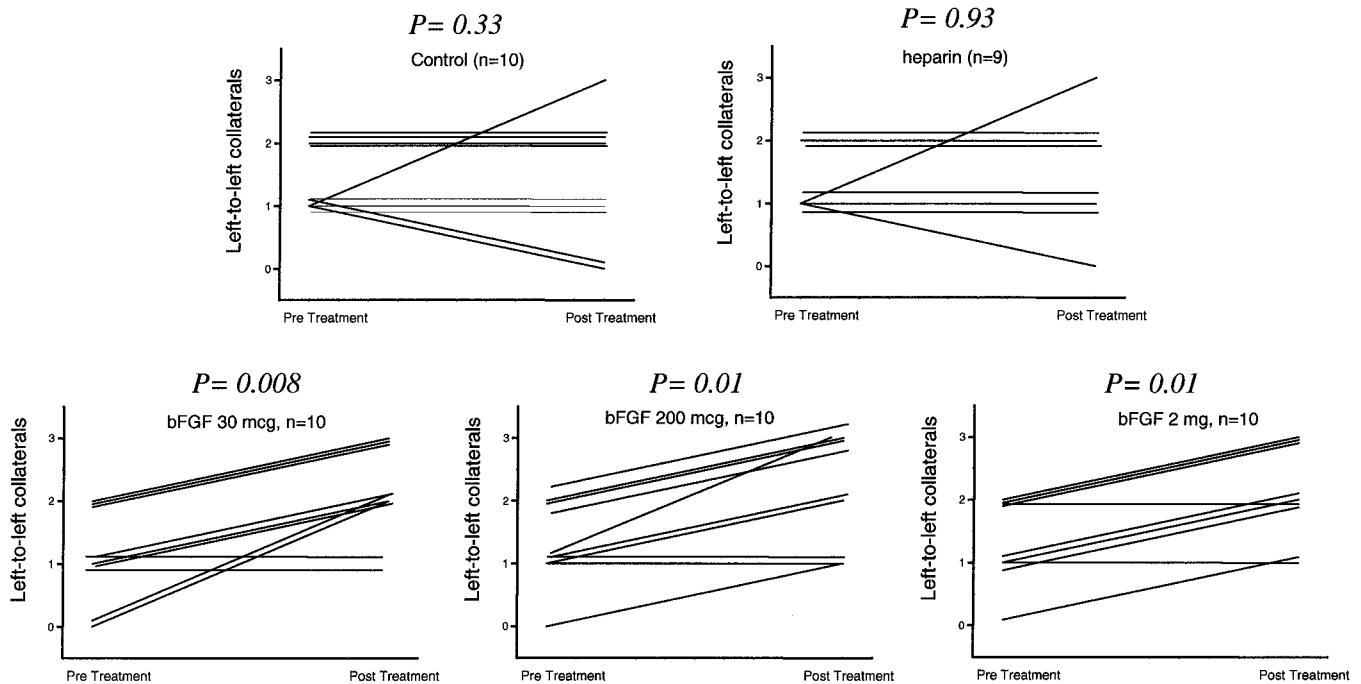


Fig. 1. Left-to-left collateral indices are plotted for controls and all three FGF-2 treatment groups. Note an increase in the left-to-left collaterals in all three FGF-2 treatment groups but not in controls.

trols), 1.25 ± 0.15 in the 200 μg FGF-2 group ($P = .002$ compared with baseline and $P = .03$ compared with controls), and 1.32 ± 0.16 in the 2 mg FGF-2 group ($P = .004$ compared with baseline and $P = .005$ compared with controls).

MRI Analysis

MRI was available on 44 animals (8 in the control group; 9 in the heparin group; and 9 in each of the 30 μg , 200 μg , and 2 mg FGF-2 groups). In five animals, MRI was not performed due to temporary technical problems with the MRI system at the time of the final study. The porcine ameroid occlusion model is associated with the development of small areas of left ventricular myocardial necrosis in most animals.

a. Global Left Ventricular Function. To assess the functional significance of FGF-2-mediated improvement in myocardial blood flow, MRI was used to assess global and regional left ventricular function in all study animals. There were no significant differences in global left ventricular function among the five groups (EF was $44.1 \pm 6.4\%$ in controls and $44.2 \pm 6.8\%$ in heparin-treated animals versus 47.07 ± 2.68 in the 30 μg FGF-2 group, 45.52 ± 3.41 in the 200 mg FGF-2 group, and 47.98 ± 3.14 in the 2 mg FGF-2 group; ANOVA, $P = .35$).

b. Regional Left Ventricular Function. Measurement of regional wall thickening in the LAD (normal territory) and LCX (ischemic) territories was used to assess regional left ventricular function (Fig. 3). LAD (normal) wall thickening was similar in all groups (ANOVA, $P = .86$). FGF-2-treated animals had improved regional wall thickening in the LCX (ischemic) territory compared with controls and heparin-treated animals [Fig. 3; LCX wall thickening (%): controls, 33.58 ± 9.91 ; heparin, 32.64 ± 13.45 ($P = .87$ compared with controls); FGF-2 30 μg , 42.12 ± 6.43 ($P = .05$ compared with controls); FGF-2 200 μg , 43.23 ± 6.41 ($P = .03$ compared with controls); and FGF-2 2 mg, 47.14 ± 3.64 ($P = .002$ compared

with controls); ANOVA, $P = .003$]. Linear regression (assuming heparin results in no significant FGF-2 release) revealed a dose-dependent improvement in LCX wall thickening in the FGF-2-treated animals ($y = 37.6 + 0.005x$, $P = .007$).

c. Myocardial Perfusion. First-pass inversion-recovery turboFLASH MRI was used to generate a space-time map of myocardial perfusion (Pearlman et al., 1995) (Fig. 4, top). Three distinct zones are observed that are characterized by either prompt signal appearance, failure of the signal to increase in intensity (infarction), or delayed signal appearance (delayed contrast arrival or ischemic zone). On the basis of contrast density data, a two-dimensional map of contrast intensity versus time was generated and was used to measure the size of the myocardial segments showing impaired (delayed) contrast arrival. Figure 4 (bottom) depicts the extent of the ischemic zone of contrast in the five groups. FGF-2 induced a dose-dependent reduction in the extent of the ischemic zone, indicating achievement of better myocardial perfusion in the FGF-2 treatment groups [Fig. 4, bottom; ischemic zone (% of left ventricle): controls, 23.54 ± 2.84 ; heparin, 22.41 ± 6.85 ($P = 0.66$ compared with controls); FGF-2 30 μg , 12.27 ± 5.82 ($P = 0.0001$ compared with controls); FGF-2 200 μg , 6.63 ± 1.97 ($P < .0001$ compared with controls); and FGF-2 2 mg, 2.02 ± 1.83 ($P < .0001$ compared with controls); ANOVA, $P < .0001$; linear regression $y = 16.7 - 0.008x$, $P < .0001$].

Histopathologic Analysis and Toxicology

There were no treatment-related macroscopic or microscopic findings in any of the organs examined. One animal had a single kidney present. There was focal to diffuse minimal thickening of the pericardium in all FGF-2 treatment groups, which was due to a slight increase in connective tissue (fibrosis). There were minimal to mild chronic inflammatory cell infiltrates accompanied by focal or multifocal

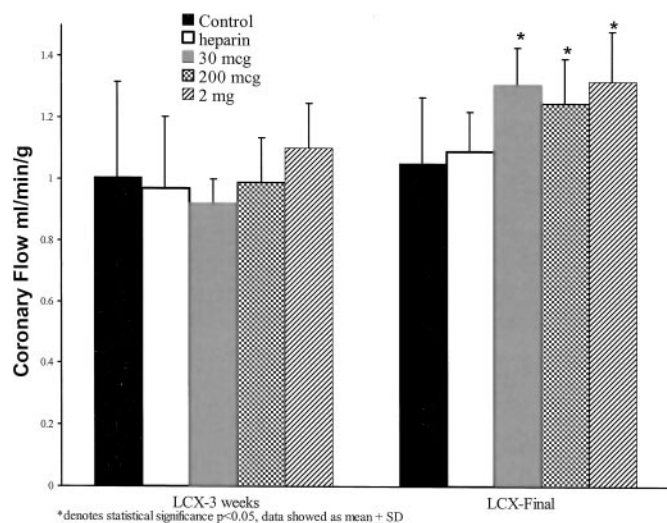


Fig. 2. Colored microspheres' derived resting LCX (collateral-dependent area) myocardial blood flow at 3 weeks (pretreatment) and at 7 weeks (the time of the final study). Controls are shown in solid black, heparin-treated animals in solid white, 30 μ g FGF-2 in gray, 200 μ g FGF-2 in checked, and 2 mg FGF-2 in striped. Coronary blood flow is increased in all FGF-2 treatment groups compared with controls and to baseline.

mineralization in all FGF-2 treatment groups. Increased vascularity was noted in the pericardium of two of three animals examined in the 200 μ g FGF-2 group and one of three animals examined in the 2 mg FGF-2 group, but was not observed in the control, heparin, or 30 μ g FGF-2 groups (Fig. 5B). In addition, the LAD and LCX in these animals were examined and they showed no evidence of intimal hyperplasia.

Finally, there was an increase in vascularity of the epicardium and myocardium in all animals from the 30 μ g, 200 μ g, and 2 mg FGF-2 groups, but not in controls or heparin-treated animals. Sections from the LCX but not the LAD distribution in all FGF-2 treatment groups showed an increase in the number of capillaries. Many of these small blood vessels were lined by endothelial cells that had large hyperchromatic nuclei, suggestive of new vascular in-growth (Fig. 5A). FGF-2 treatment did not result in any significant abnormalities in serum chemistries, hematology, and coagulation studies.

Discussion

Several studies have demonstrated that chronic administration of FGF-2 (Yanagisawa-Miwa et al., 1992; Battler et al., 1993; Harada et al., 1994; Unger et al., 1994; Landau et al., 1995; Lazarous et al., 1995; Uchida et al., 1995) or VEGF (Banai et al., 1994; Pearlman et al., 1995; Engler, 1996; Harada et al., 1996) results in significant myocardial angiogenesis in animal models of myocardial ischemia and infarction. However, because of the protracted time course required for new collateral vessel development, many attempts to stimulate myocardial angiogenesis have used methods of prolonged growth factor delivery, including gene therapy, continuous infusions, repeated injections, and sustained release polymers. Many of these therapeutic strategies, particularly those requiring repeated access or major surgical intervention, are impractical from a clinical standpoint. The pericardial space offers potentially unique advantages in conve-

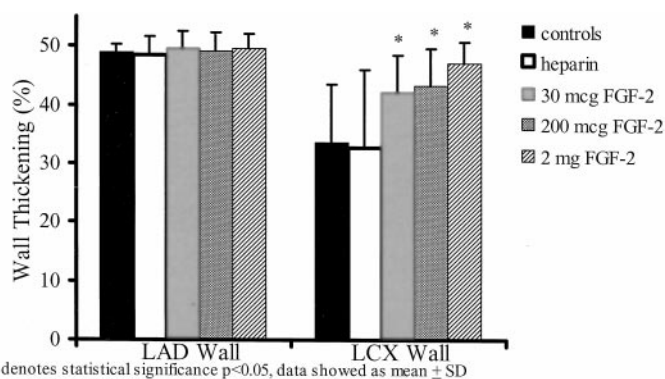


Fig. 3. Regional wall thickening in the LAD (normal) and LCX (collateral-dependent) distribution. There was a dose-dependent improvement in regional wall motion with FGF-2 treatment compared with controls. Note that LCX wall motion in the 2 mg FGF-2 group was near normalized (to LAD motion).

nience, safety, and efficacy as a cardiovascular drug depot site for the administration of proangiogenic growth factors.

This study was designed to investigate the effects of a single intrapericardial injection of increasing FGF-2 doses in a porcine model of chronic myocardial ischemia. Separate saline and heparin control arms were used to address the potential angiogenic effects of heparin alone or in combination with FGF-2 (Norrby, 1993; Rosenberg et al., 1997). However, no significant differences were found between the heparin (at the dose used) and saline arms in any of the measured parameters. Intrapericardial FGF-2, on the other hand, resulted in an improvement in left-to-left angiographic collaterals, occluded LCX coronary artery blood flow, LCX (ischemic territory) myocardial perfusion, and LCX regional wall function as measured by MRI. Improvements in ischemic territory regional wall function and myocardial perfusion were positively correlated with FGF-2 dose, with near normalization of wall function and perfusion in the 2 mg FGF-2 group. Qualitative histopathologic examination showed increased myocardial vascularity in FGF-2-treated animals without any adverse findings.

In considering growth factor-induced neovascularization, it is important to distinguish intramyocardial collateral development from formation of epicardial collaterals (neoarteriogenesis). The process of intramyocardial collateral development (angiogenesis) is characterized by appearance of thin-walled vessels with poorly developed tunica media generally under 200 μ m in diameter and by an increase in the number of true capillaries (<20 μ m in diameter containing only a single endothelial layer), whereas the neoarteriogenesis is characterized by development of larger vessels (>200 μ m in diameter) with well developed tunica media and adventitia that usually form close to the site of the occlusion of a major epicardial coronary artery (bridging collaterals) or extend from one coronary artery to the other (Schaper, 1996). The distinction between these two groups of newly formed vessels is important not only from the point of view of their location but also because stimuli for their development appear to be quite different and because they may exhibit different physiological properties. It is unclear whether intrapericardially administered FGF-2 exerts its beneficial effects on myocardial revascularization by acting on the epicar-



Fig. 4. Top, MRI perfusion images showing a long axis view of the left ventricle. After the i.v. injection of gadodiamide, contrast appears first in the right ventricle (solid arrows), then the left ventricle (open arrows), then the left ventricular myocardium (arrow heads). Bottom, ischemic zone (delayed contrast arrival zone) extent in all groups demonstrates a dose-dependent reduction in the extent of the ischemic zone in FGF-2-treated animals.

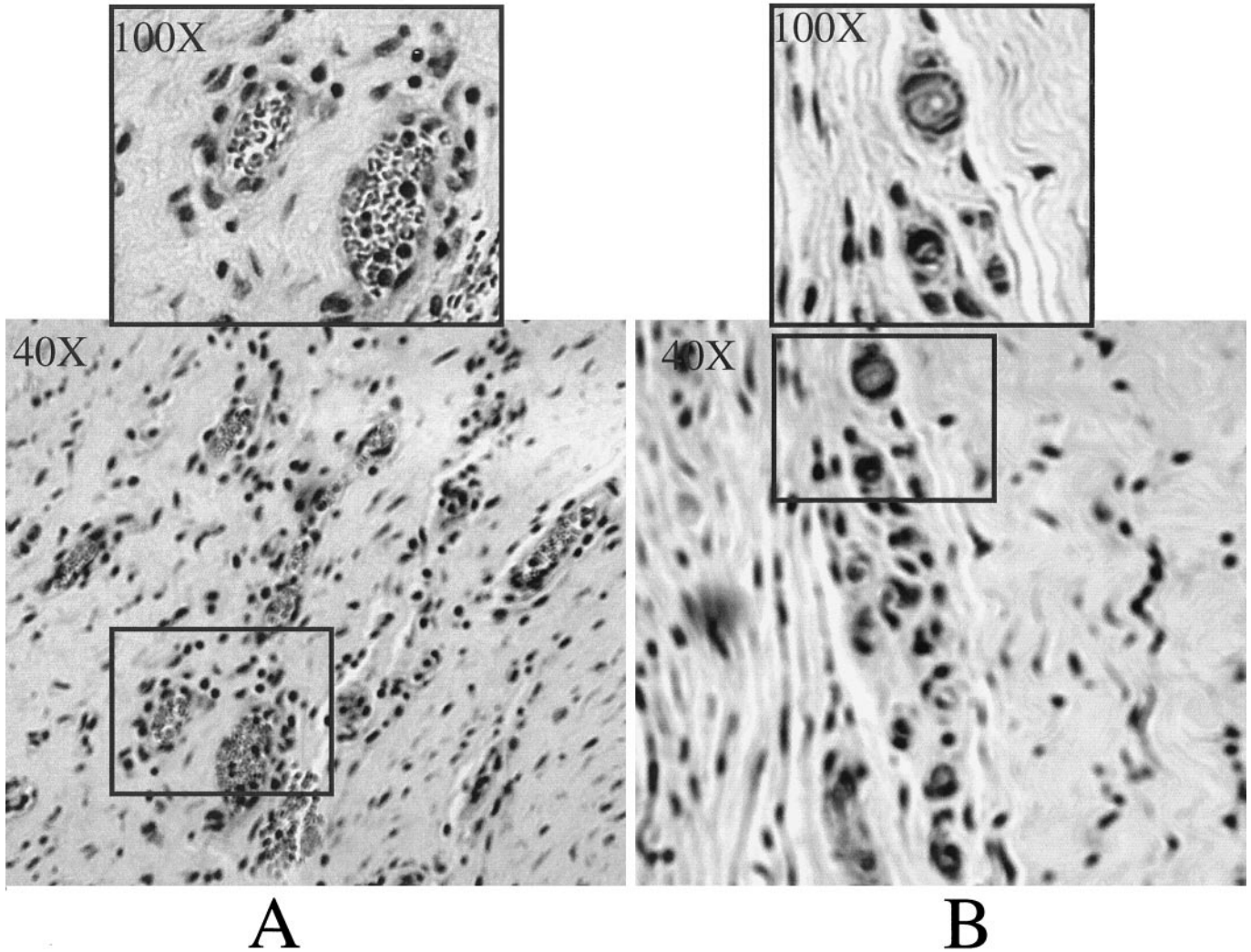
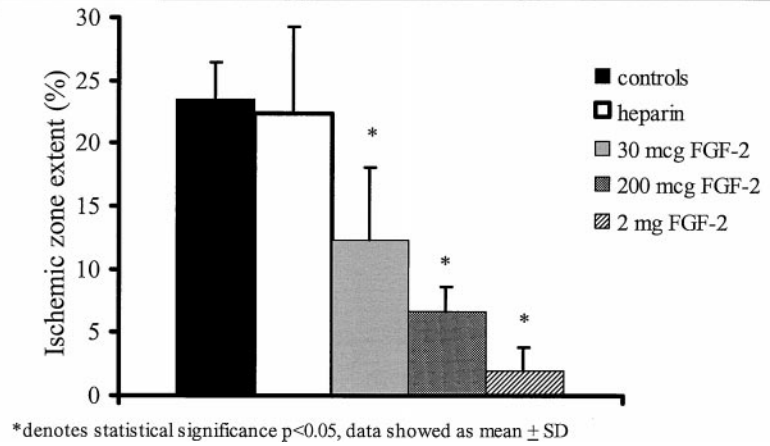


Fig. 5. A, histopathologic sections from the LCX distribution demonstrate an increased number of capillaries in all treatment groups. Many of these small blood vessels are lined by endothelial cells with large hyperchromatic nuclei, suggesting new vascular in-growth. B, increased vascularity was noted in the pericardium of two of three animals examined in the 200- μ g group and one of three animals examined in the 2-mg group.

dial surface (where it is in greatest concentration) to induce collateralization around sites of occlusion in the epicardially situated major coronary arteries, or whether it diffuses into the myocardium and myocardial microcirculation to induce angiogenesis at a more microscopic level, or both. However, the demonstrated effectiveness of the low-dose (30 μg) intrapericardial FGF-2 suggests that the presence of FGF-2 on the epicardial surface may play a key role in inducing functionally significant angiogenesis.

The present study is the first study to demonstrate functionally significant angiogenesis in a model of chronic myocardial ischemia using a single intrapericardial injection of FGF-2. We have previously shown that FGF-2 (10–100 μg) incorporated into heparin-alginate microspheres (for sustained delivery) and implanted on the epicardial surface of the occluded LCX results in significant improvement in myocardial function in the setting of chronic myocardial ischemia (Harada et al., 1994; Lopez et al., 1997). This delivery method, however, may not be practical for the majority of patients with coronary artery disease, making the single intrapericardial injection approach a potentially more attractive strategy for therapeutic myocardial angiogenesis. Of note, in the current study, intrapericardial FGF-2-induced improvements in measured parameters comparable with epicardially implanted FGF-2/heparin-alginate microspheres. Thus, the pericardial space may provide a unique drug delivery option for therapeutic myocardial angiogenesis.

Landau and colleagues (Landau et al., 1995) have previously demonstrated a localized angiogenic response to intrapericardial FGF-2 in a rabbit model of angiotensin II-induced cardiac hypertrophy, although the use of an intrapericardial infusion with an osmotic pump and the cardiac hypertrophy model limits its applicability and comparability to this study. Uchida et al. (1995) have studied the effect of intrapericardial FGF-2 (30 μg FGF-2 + 3 mg heparin) in a canine model of acute myocardial infarction and have demonstrated an angiogenic response with FGF-2 treatment using infarct mass and histopathology as outcome measures. However, the model we describe is one of chronic myocardial ischemia with occasional small infarcts ($2.6 \pm 3.7\%$). Indeed, this model may reflect cardiac hibernation with viable but underperfused myocardium suffering recurrent ischemia during daily activity, leading to depressed regional myocardial function without evidence of myocardial necrosis. This depressed regional wall motion is improved by FGF-2 treatment, which reflects revascularization and restoration of near-normal blood flow to the chronically ischemic myocardium.

A potential limitation to the use of the FGF-2 in patients with coronary atherosclerosis and saphenous vein bypass grafts is the potential exacerbation of intimal hyperplasia and progression of coronary atherosclerosis (Edelman et al., 1992; Sterpetti et al., 1996). However, we did not find any evidence of intimal hyperplasia or coronary atherosclerosis in our animal model in concordance with previous studies (Lazarous et al., 1996). This and previous models, nonetheless, are limited by the use of FGF-2 in normal (noninjured) coronary arteries in normolipemic animals. The pericardial instrumentation at the time of ameroid placement is one limitation to this study; the minimal

pericardial thickening may have affected the distribution and pharmacokinetics of FGF-2 administration.

Conclusion. We conclude that a single intrapericardial injection of FGF-2 + heparin in a porcine model of chronic myocardial ischemia results in a significant increase in angiographic collaterals and blood flow in an experimentally occluded coronary artery. These benefits were accompanied by improvements in myocardial perfusion and function in the ischemic territory, as well as histologic evidence of increased myocardial vascularity. No adverse effects of FGF-2 were observed. Single bolus intrapericardial FGF-2 administration may prove to be a useful therapeutic strategy for the treatment of patients with ischemic heart disease.

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