Intracoronary Basic Fibroblast Growth Factor Enhances Myocardial Collateral Perfusion in Dogs

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OBJECTIVES

In preparation for clinical trials of basic fibroblast growth factor (bFGF) to treat ischemic heart disease, we sought to identify a clinically feasible method of bFGF administration.

BACKGROUND

Basic FGF has been shown to promote collateral development after experimentally induced coronary occlusion; however, methods of bFGF delivery that have been shown to be effective in previous investigations would not be practical for clinical use.

METHODS

Four randomized, blinded, controlled investigations were conducted independently and sequentially in an established canine model. For all studies, dogs underwent operative placement of proximal left circumflex coronary artery ameroid constrictors. The four investigational regimens included: 1) bFGF by central venous bolus injection, 1,740 μg/day for one, two or seven days; 2) bFGF by intravenous infusion, 100 μg/kg body weight per day for seven days; 3) bFGF by pericardial instillation, 2,000 μg/day for 7 days; and 4) bFGF by intracoronary injection (Judkin’s technique), 100 μg/kg per day for one or two days. Each substudy included a contemporaneous vehicle control group. Collateral perfusion (microspheres) was assessed during maximal coronary vasodilation during the first month after ameroid placement.

RESULTS

Maximal collateral perfusion in dogs that received intracoronary bFGF for two days exceeded that of concurrent control dogs by 31% (p < 0.01). Perfusion was not increased in dogs that received single-dose intracoronary bFGF. Basic FGF administration by central venous bolus injection, intravenous infusion and pericardial injection failed to enhance collateral perfusion.

CONCLUSIONS

Administration of bFGF by the intracoronary route, an intervention that is feasible in patients, augments collateral development in dogs. These data provide a rationale for clinical testing of intracoronary bFGF in ischemic heart disease. (J Am Coll Cardiol 2000;35:519–26) © 2000 by the American College of Cardiology

The facilitation of coronary collateral growth represents a novel and potentially important therapeutic approach for patients with ischemic heart disease. A number of angiogenic growth factors have been evaluated experimentally as agents to promote vascular development. Two of the most widely studied agents, basic fibroblast growth factor (bFGF) and vascular endothelial growth factor, have been shown to promote collateral development in animal models of myocardial ischemia (1–5). Both agents are undergoing clinical trials in the U.S. (6). Acidic fibroblast growth factor has received somewhat less attention in preclinical studies, but has been reported to increase angiographically visible collateral vessels in patients undergoing surgical coronary revascularization (7). Although there are now numerous reports suggesting salutary biologic effects of angiogenic growth factors in the setting of experimental myocardial ischemia, no simple, clinically feasible method of growth factor protein delivery has emerged as an effective means to promote collateral development. By and large, the methods used to administer growth factors in these studies would not be practical in patients with ischemic heart disease. Moreover, disparities in species, experimental models, doses and end points obfuscate meaningful comparison of the published data. In preparation for clinical trials, we attempted to identify an optimal method of growth factor delivery for promotion of myocardial collateral development. We evaluated the biologic effects of bFGF using four clinically...
plausible routes of administration: 1) central venous bolus injection; 2) peripheral intravenous (IV) infusion; 3) pericardial instillation; and 4) intracoronary (IC) injection, the last using standard catheter-based delivery. A secondary aim of the study was to determine, when possible, the minimal effective course of bFGF treatment for each delivery route. To improve comparability across these studies, we synchronized bFGF administration relative to the ischemic stimulus and used similar bFGF doses with each route of administration.

METHODS

Four substudies were carried out independently and sequentially between November 1993 and June 1997. All procedures were conducted in accordance with institutional ethical guidelines (8). One hundred and four purpose-bred dogs were used for the studies.

General methods. The surgical preparation of this model has been described previously (4). Anesthesia was induced with acepromazine, 0.2 mg/kg body weight intramuscularly (IM), and maintained with thiopental sodium, 15 mg/kg IV, and methoxyflurane. A thoracotomy was performed through the left fifth intercostal space using aseptic technique. The pericardium was incised, the proximal left circumflex coronary artery (LCx) was dissected and a 2.0- to 2.5-mm ameroid constrictor (Research Instruments Manufacturing, Corvallis, Oregon) was applied proximal to the first marginal branch. A hydraulic balloon occluder was secured around the vessel immediately distal to the ameroid. A Silastic catheter was inserted into the left atrial (LA) appendage. Its injection port was buried in the subcutaneous tissue of the interscapular area, accessible for microsphere injections. The pericardium and chest were closed, and the animals received postoperative care with aseptic technique. A Silastic catheter was inserted into the left atrial (LA) appendage. Its injection port was buried in the subcutaneous tissue of the interscapular area, accessible for microsphere injections. The pericardium and chest were closed, and the animals received postoperative care with aseptic technique.

Figure 1. Summary of experimental designs and schedules for perfusion measurements. Solid squares indicate bFGF doses administered; open squares indicate injection of vehicle. Daily bFGF doses by substudy: central venous: 1,740 µg/day; peripheral IV: 100 µg/kg per day; pericardial: 2,000 µg/day; IC: 100 µg/kg per day. Timing of perfusion measurements denoted by X. N represents number of animals with evaluable data in each cohort.

Recombinant human bFGF was provided by Scios, Inc. (Mountainview, California). Aliquots of bFGF and equivalent volumes of saline were numerically coded and stored at −20°C until use. Aliquots were diluted with 0.5 ml of citrate buffer immediately before administration. Weekly laboratory studies included a complete blood count, platelet count and levels of blood urea nitrogen, creatinine, glucose, total bilirubin, albumin, serum glutamic-oxaloacetic transaminase, serum glutamic-pyruvic transaminase, alkaline phosphatase, creatine phosphokinase, calcium and phosphate.

Central venous substudy. Thirty-two dogs were used for this study. Catheters were implanted in the right atrial (RA) appendage. Its injection port was buried in the subcutaneous tissue of the interscapular area, accessible for microsphere injections. The pericardium and chest were closed, and the animals received postoperative care with aseptic technique. Intravenous lidocaine was administered prophylactically, and procainamide, 500 mg orally twice daily for seven days, was administered at the discretion of the veterinary staff. An oral broad-spectrum antibiotic was given twice daily for seven days. Catheters were flushed with 2 to 3 ml of heparinized saline (2 U/ml) before and after test drug administration to maintain patency.

Peripheral IV substudy. The peripheral IV study included 25 dogs. Dogs assigned to bFGF treatment received daily infusion of 100 µg/kg of bFGF for 7 days, 10 to 16 days after ameroid placement (Fig. 1). The control group served
also as a control group for a concurrent investigation (unpublished data) in which an alternate active drug was administered orally. Approximately half of the control dogs received the vehicle as a daily infusion; half received no treatment. Appropriate volumes of coded test drugs were added to 500 ml of 0.9% saline and infused intravenously over 3 to 4 h through the external jugular or cephalic vein.

**Pericardial substudy.** Fifteen dogs were used for this substudy. During surgical ameroid placement, the pericardiotomy incision was limited to ~2 cm in length. Numerous side holes were placed in the terminal 2 cm of a 6.6F Silastic end hole catheter (Bard Access Systems, Salt Lake City, Utah), and the distal catheter was secured in the pericardium with a pursestring suture. The pericardium was carefully sealed to prevent leakage, and the catheter infusion port was secured subcutaneously. In a pilot dog, not included in the final analyses, the integrity of the pericardial sac was evaluated 10 days after the operation by injecting a contrast agent into the pericardial catheter under fluoroscopic guidance. This demonstrated free flow of contrast agent within the pericardium without leakage. Dogs were randomized to receive bFGF, 2,000 μg/day for seven days, or vehicle during the period 10 to 16 days after ameroid placement (Fig. 1).

**Intracoronary substudy.** Thirty-two dogs were used for the IC substudy. Appropriate volumes of test drugs were administered into the left main coronary artery during cardiac catheterization using standard the Judkin’s technique. The dogs were randomized to three cohorts: bFGF 100 μg/kg × 1 (day 16); bFGF 100 μg/kg × 2 (days 14 and 16); or vehicle on one of these two schedules (Fig. 1). Before catheterization, the dogs were briefly anesthetized with a short-acting barbiturate, intubated and mechanically ventilated. Anesthesia was maintained with isoflurane. Blood pressure and an electrocardiogram (ECG) were monitored throughout the procedure. The groin was shaved and prepped, and a 5F Cordis introducer catheter was inserted percutaneously in the femoral artery. Cardiac catheterization was performed in standard fashion. Contrast material (hexabrix, 3 to 5 ml, diluted 1:1) was injected to ensure engagement of the left main coronary artery. Occlusion of the LCx was confirmed by a lack of anterograde flow beyond the ameroid constrictor. Test drugs were injected over 2 min. The catheter was flushed with saline before and after test drug injection, and an additional contrast injection was performed after test drug injection to document maintenance of the catheter’s position. Heparin was not used during catheterization.

**Randomization, blinding and end points.** Blocked randomization was used in all studies. Investigators responsible for surgical instrumentation, animal care, test agent and microsphere injections, data analysis and interpretation had no knowledge of treatment group. Technical problems precluded data interpretation in some animals; decisions to exclude nonevaluable data were made without knowledge of treatment assignment. For all studies, the primary prospectively defined end points were maximal coronary conductance in the collateral-dependent zone (the quotient of perfusion and arterial pressure) and the ischemic zone/normal zone (IZ/NZ) perfusion ratio. Regional myocardial perfusion was quantified during pharmacologically induced maximal coronary vasodilation. Maximal vascular conductance (perfusion divided by mean arterial pressure) was selected as the primary surrogate of collateral development because it could be assessed: 1) in the conscious state, in the absence of confounding influences of anesthesia or surgery, or both; 2) on a serial basis; and 3) during reproducible vasodilatory stress.

**Study procedures.** The dogs were sedated with acepromazine, 0.1 to 0.25 mg/kg IM, or diazepam, 1 to 2 mg/kg IV, or both. Using local lidocaine anesthesia, a 5F Cordis introducer catheter was inserted into the left or right femoral artery for reference blood sample withdrawal. Arterial blood pressure was measured through the catheter side port, and the ECG was continuously recorded. Maximal coronary vasodilation was induced with chromonar hydrochloride, 8 mg/kg, infused through the LA catheter over 20 to 30 min. Regional perfusion was assessed using the reference sample technique, as we have done previously (1,2,4,5). Microspheres were randomly selected from four isotopes (New England Nuclear, Boston, Massachusetts; central venous and IC studies) or eight dual-fluorescent labels (Triton Technology, San Diego, California; peripheral IV and pericardial studies). Approximately 3 to 4 × 10^6 microspheres, 15 μm in diameter, were injected into the LA catheter with simultaneous withdrawal of a femoral artery reference sample at 7.75 ml/min. Complete LCx occlusion requires ~10 to 20 days after ameroid placement. Therefore, during assessment of collateral perfusion on days 10 and 17, the hydraulic balloon occluder was temporarily inflated 15 s before the injection of microspheres to arrest residual anterograde LCx flow. The occluder was permanently inflated on day 24 to ensure timely and complete LCx occlusion. The schedule for determination of regional myocardial perfusion for all studies is summarized in Figure 1. The final myocardial perfusion assessments in the collateral-dependent zone (IZ/NZ) perfusion ratio. Regional myocardial perfusion was quantified during pharmacologically induced maximal coronary vasodilation. Maximal vascular conductance (perfusion divided by mean arterial pressure) was selected as the primary surrogate of collateral development because it could be assessed: 1) in the conscious state, in the absence of confounding influences of anesthesia or surgery, or both; 2) on a serial basis; and 3) during reproducible vasodilatory stress.

**Calculation of myocardial perfusion.** For studies using radiolabeled microspheres, the hearts were analyzed as previously described (1,2). For experiments in which fluorescent microspheres were used, a single 7-mm left ventric-
ular slice was divided into 16 samples, which were digested and analyzed by Interactive Medical Technologies (La Jolla, California).

**Infarct size.** Infarct size was formally quantified in the central venous substudy. The short-axis slice basal to the two central slices was analyzed. Radial wedges were paraffin-embedded and stained using Masson’s trichrome method to differentially stain viable myocardium (red) versus scar (blue). The sections were examined in their entirety using a microscope fitted with a 1x objective lens. Rectangular fields were digitized in true color, and areas of scarred myocardium were quantified using computer-based image analysis. Total tissue area was determined using a macro lens, and infarct area was computed as the quotient of scarred area/total tissue area x 100%. For other substudies, infarct size was estimated by inspection of the fixed slices to identify dogs with extensive infarcts.

**Statistical methods.** Continuous data are summarized as the mean value ± SE. Differences between the mean values of two groups were analyzed using the Student t test for unpaired data (two-tailed). For the IC substudy, there were two active treatment groups and one control group. Differences between the mean values of three groups were assessed using single-factor analysis of variance followed by individual t tests and the Bonferroni correction. Thus, there were three salient comparisons (bFGF ×1 vs. control; bFGF ×2 vs. control; bFGF ×2 vs. bFGF ×1), and p values were multiplied by a factor of 3, with alpha set at 0.05. Relative differences between two groups are expressed as mean percent differences ± 95% confidence interval (CI).

**RESULTS**

Evaluable perfusion data were obtained from 93 (89%) of 104 dogs. Seven deaths occurred before treatment assignment: three deaths were within the first two weeks after the operation, presumed secondary to abrupt or premature amniotic–induced L CXs occlusion; two deaths were attributed to anesthesia after cardiac catheterization in the IC study; and two deaths were related to inadvertent lidocaine overdose in the IV study. Four dogs were excluded after perfusion data were obtained—two because of technical problems and two because of unusually extensive infarcts.

Generally, bFGF was well tolerated by dogs that received only one or two doses of the peptide by any route; however, anorexia, vomiting and excessive thirst were observed by the veterinary staff in the majority of dogs after repeated administration of the 100 μg/kg dose of bFGF.

**Hemodynamic data.** Systemic hemodynamic data were obtained 10, 17, 24 and 38 days after amniotic placement in the central venous study. There were no significant changes in mean arterial pressure or heart rate with respect to time and no significant intergroup differences (data not shown). In the peripheral IV study, heart rate and mean arterial pressure were similar in the two groups before treatment (day 10). At end-treatment (day 17), there was a significant reduction in systolic blood pressure in bFGF-treated dogs (−12%; 95% CI −3% to −22%), associated with an increase in heart rate (32%; 95% CI 10% to 54%). Interestingly, bFGF–treated dogs exhibited sustained reductions in mean arterial pressure up to three weeks after the conclusion of bFGF treatment. Reductions of 17 ± 11%, 20 ± 12% and 13 ± 10% were observed on days 24, 31 and 38, respectively (mean percent decrease ± 95% CI). Systolic and diastolic blood pressures were proportionately decreased. Hemodynamic measurements were obtained only at the end of the pericardial and IC studies, and there were no significant differences between groups (data not shown). The early hemodynamic response to bFGF administration was not assessed.

**Infarct size.** Three dogs in the central venous substudy had significant infarcts, ~18%, 15% and 6% in area, whereas the remainder of the dogs had insignificant scarring, <2% of the left ventricle, and generally <1%. There was no relation between treatment group and infarct size. One dog in each of the peripheral IV and IC substudies had notable infarcts. Both animals were excluded from further analyses.

**Collateral perfusion.** The IZ and NZ conductances for the central venous and peripheral IV substudies are summarized in Tables 1 and 2, respectively. In both substudies, maximal NZ conductance was similar in all groups and did not change significantly over time. Maximal IZ conductance and IZ/NZ perfusion ratios increased in all groups with respect to time; however, there were no significant intergroup differences. In the pericardial substudy, there were no differences between bFGF- and saline-treated dogs with respect to maximal IZ conductance or IZ/NZ ratios (Table 3). For the IC substudy (Table 4), maximal NZ conductance was similar in all groups. Mean IZ/NZ perfusion ratios were 0.45 ± 0.02, 0.36 ± 0.03 and 0.34 ± 0.02 in the bFGF ×2, bFGF ×1 and vehicle cohorts, respectively. Analysis of variance was significant at p = 0.0084, with significant differences between the bFGF ×2 and control groups (p = 0.0024) and the bFGF ×2 and bFGF ×1 groups (p = 0.017). After application of the Bonferroni correction, the difference between the bFGF ×2 and control groups remained highly statistically significant (p = 0.0072), whereas the difference between the bFGF ×2 and bFGF ×1 groups did not (p = 0.051). For comparison between the bFGF ×2 and control groups, the relative percent difference in perfusion ratios was 31% (95% CI 12% to 49%).

**Laboratory data.** Administration of one or two bFGF doses by IC or central venous routes was not associated with significant hematologic or biochemical abnormalities. Significant toxicity was limited to cohorts that received seven-dose bFGF regimens by the central venous, IV or pericardial routes. As in previous studies (4,5), the most pronounced effects were hematologic. Transient thrombocytopenia and
### Table 1. Coronary Collateral Conductance and the IZ/NZ Perfusion Ratio (Central Venous Study)

<table>
<thead>
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<th>Day 10</th>
<th>Day 17</th>
<th>Day 24</th>
<th>Day 38</th>
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<td>Conductance</td>
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<td></td>
<td>IZ</td>
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<tr>
<td>Group 0</td>
<td></td>
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<tr>
<td>(n = 7)</td>
<td>0.28 ± 0.06</td>
<td>4.26 ± 0.92</td>
<td>0.09 ± 0.03</td>
<td>1.15 ± 0.3</td>
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<td>Group 1</td>
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<td>(n = 8)</td>
<td>0.18 ± 0.03</td>
<td>3.43 ± 0.50</td>
<td>0.07 ± 0.02</td>
<td>0.97 ± 0.17</td>
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<td>Group 2</td>
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<tr>
<td>(n = 8)</td>
<td>0.46 ± 0.11</td>
<td>5.06 ± 0.94</td>
<td>0.11 ± 0.03</td>
<td>1.33 ± 0.37</td>
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<td>Group 7</td>
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<tr>
<td>(n = 7)</td>
<td>0.42 ± 0.14</td>
<td>4.22 ± 0.49</td>
<td>0.12 ± 0.04</td>
<td>1.13 ± 0.29</td>
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*Conductance is expressed as ml/min per 100 g per mm Hg. Ischemic zone (IZ) and normal zone (NZ) coronary conductance and the IZ/NZ ratio are with respect to time in group 0 (control group), group 1 (bFGF ×1), group 2 (bFGF ×2) and group 7 (bFGF ×7). Data are presented as the mean value ± SEM.

### Table 2. Coronary Collateral Conductance and the IZ/NZ Perfusion Ratio (Peripheral IV Study)

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<th>Day 24</th>
<th>Day 31</th>
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<td>IZ</td>
<td>NZ</td>
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<td>NZ</td>
<td>IZ</td>
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<tr>
<td>bFGF (n = 10)</td>
<td>0.12 ± 0.03</td>
<td>5.34 ± 0.63</td>
<td>0.02 ± 0.00</td>
<td>1.23 ± 0.18</td>
<td>4.94 ± 0.40</td>
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<tr>
<td>Saline (n = 10)</td>
<td>0.14 ± 0.03</td>
<td>5.13 ± 0.44</td>
<td>0.03 ± 0.01</td>
<td>0.79 ± 0.13</td>
<td>4.95 ± 0.50</td>
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</tbody>
</table>

*Conductance is expressed as ml/min per 100 g per mm Hg. Ischemic zone (IZ) and normal zone (NZ) coronary conductance and the IZ/NZ ratio are with respect to time in basic fibroblast growth factor (bFGF)- and saline-treated dogs. Data are presented as the mean value ± SEM.
anemia were observed after bFGF ×7 doses by both the central venous and peripheral IV routes, although they were not observed with the pericardial route. For dogs that received central venous bFGF ×7 doses, the platelet count decreased from 240 ± 17 × 10^3/μl before treatment to 150 ± 24 × 10^3/μl during the week of treatment, normalizing one week later. Thrombocytopenia was quantitatively similar with peripheral IV bFGF. A decrease in hemoglobin was observed one week after cessation of treatment in dogs that received central venous bFGF ×7 doses (mean decrease ≈ 2.8 g/dl to a nadir of 11.8 ± 0.4 g/dl), with partial recovery during the next two weeks. Anemia was also associated with the seven-dose peripheral IV bFGF regimen, although the nadir in hemoglobin was less pronounced (13.7 ± 0.4 g/dl vs. 13.5 ± 0.4 g/dl, bFGF vs. control, p < 0.02) and delayed until the final week of the study. Moderate leukocytosis was observed in dogs that received pericardial IV and pericardial bFGF, although the changes reached statistical significance only in the pericardial substudy. In the peripheral IV substudy, leukocytosis peaked the week after IV bFGF administration and was largely due to granulocytosis and monocytosis (peak white blood cell count 17.3 ± 2.3 vs. 12.2 ± 1.2 × 10^3/μl, bFGF vs. control group, p = 0.053). Similar changes in leukocyte count were observed in the dogs that received pericardial bFGF (peak white blood cell count during treatment 17.0 ± 1.7 vs. 12.3 ± 0.5 × 10^3/μl, bFGF vs. control group, p < 0.05), with no apparent difference thereafter. Pericardial bFGF administration was associated with transient hypoalbuminemia and elevated serum glutamic-pyruvic transaminase during the week after treatment. The nadir in serum albumin was 2.5 ± 0.1 g/dl (vs. 3.0 ± 0.1 g/dl in control group, p < 0.005), with a peak serum glutamic-pyruvic transaminase level of 103 ± 16 (vs. 39 ± 16 IU/liter in control group, p < 0.02).

**DISCUSSION**

A number of investigations have suggested that angiogenic growth factors can enhance collateral function in the setting of experimental coronary artery occlusion; however, the methods of growth factor protein administration used to date are not well suited for routine clinical use. This investigation provides the first evidence, to our knowledge, that recombinant growth factor protein, administered through a clinically practical route, can enhance myocardial collateral development in vivo. Although bFGF by the IC route improved collateral perfusion, it was ineffective when administered by the peripheral or central venous routes or when administered by the intrapericardial route.

We previously demonstrated that IC bFGF (1) and vascular endothelial growth factor (2) promote coronary collateral development in a similar model; however, multiple doses of the growth factors were instilled directly into the collateral-dependent area, distal to the point of coronary occlusion, a near impossibility in patients with coronary disease. More recently, we found that bFGF improved collateral perfusion when administered repeatedly by a systemic arterial route (LA injection) (4,5), but repeated LA access represents a major, practical obstacle. Other investigators have studied sustained-release polymers for local growth factor delivery (3). These polymers have potential for adjunctive use during the course of surgical coronary revascularization, but presently require invasive techniques for deployment.

**Intravenous bFGF.** Basic FGF is a heparin-binding growth factor that binds avidly to glycosaminoglycans in vascular subendothelial matrix (10). Given the vast cross-sectional area of the pulmonary circulation, there exists the potential for substantial first-pass lung uptake of intravenously administered bFGF. When we recapitulated our previous systemic arterial study (5), shifting only the site of bFGF administration from the LA to the RA, there was no measurable effect on collateral function. Given the important clinical ramifications of these findings, and in recognition of the limited statistical power of the central venous substudy, the peripheral IV substudy was performed as a confirmatory investigation. Again, there was no evidence of a salutary effect. Our ability to replicate these findings in independent but related studies suggests that it is unlikely that a biologically meaningful effect was missed by chance. Anemia and thrombocytopenia were observed in bFGF-treated cohorts in both of these substudies, quantitatively similar to those incidences observed in the previous systemic arterial investigation (5). Thus, for intravenous bFGF, there was no evidence of a beneficial effect, even at doses associated with hematologic toxicity.

Previously, we observed both short- and long-term blood pressure–lowering effects of bFGF (4). Only long-term...
changes were assessed in the present investigation. Although a prolonged decrease in blood pressure was observed in bFGF-treated dogs in the peripheral IV substudy, a long-term hemodynamic effect was not detected in the central venous substudy. The explanation for this disparity is unknown.

**Pericardial bFGF.** The pericardial space has been considered as a target for gene transfer of angiogenic growth factors (11,12). Intrapericardial bFGF has been reported to induce localized increases in myocardial vascularity in rabbits (13) and to reduce infarct size in a canine model (14). Conceptually, the pericardium could function as a paracrine organ, storing and releasing bFGF to the developing collateral circulation. Recently, we calculated that 19% of a pericardial dose of radiolabeled bFGF could be recovered from the myocardium after 150 min (15), suggesting that this route would be effective. Our findings, however, are counter to this prediction. This may relate to the manner of bFGF presentation to vascular cells or the potential for pericardial inflammatory mediators to cause peptide degradation, or both. Consistent with the latter hypothesis, elevated peripheral polymorphonuclear leukocyte counts were observed in bFGF-treated dogs in the pericardial substudy, suggesting a proinflammatory state. Moreover, this premise is supported by the fact that intrapericardial bFGF did not induce blood pressure lowering, anemia or thrombocytopenia characteristic of systemic arterial and intravenous bFGF administration.

Ostensibly, our findings appear inconsistent with those of Landau et al. (13) and Uchida et al. (14). The former study assessed the effect of pericardial bFGF infusion during and after IV angiotensin II treatment (13). Angiotensin II was administered to induce left ventricular hypertrophy, thereby decreasing capillary density and inducing ischemia. Localized increases in epicardial vascularity were observed with bFGF administration. Uchida et al. reported a decreased infarct size associated with intrapericardial administration of bFGF in a canine model of inorganic mercury left anterior descending coronary artery embolization (14). Neither of these studies were designed to evaluate collateral development or collateral perfusion, however, and it would be an overstatement to conclude that our results are discordant with either.

**Intracoronary bFGF.** The bFGF dose was normalized to body mass (100 μg/kg) for the IC substudy, approximating the doses given in previous investigations (4,5). Administration of two but not one 100 μg/kg bFGF dose was associated with significant enhancement of collateral perfusion. Parenthetically, it should be noted that the IZ/NZ ratio for the IC substudy control group is somewhat lower than that of the control groups in the other substudies, likely because the final perfusion assessment was made on day 33 in the IC substudy, whereas it was made later (on day 38) in the other substudies. The mean day 33 IZ/NZ ratio observed in IC substudy control animals is virtually identical to that found in control animals at a similar time point (day 31) in previous studies (4).

| Table 4. Coronary Collateral Conductance and the IZ/NZ Perfusion Ratio (IC Study) |
|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
| Control Group                  | bFGF × 1 Dose                  | bFGF × 2 Doses                  |
|                                | Conductance                    | Conductance                    | Conductance                    |
| IZ/NZ                          | MAP†                           | IZ/NZ                          | MAP†                           |
| IZ                              | 1.28 ± 0.13                    | 1.62 ± 0.04                    | 1.07 ± 0.02                    |
| NZ                              | 4.97 ± 0.44                    | 4.91 ± 0.44                    | 4.49 ± 0.44                    |
| IZ                              | 1.86 ± 0.16                    | 1.93 ± 0.16                    | 2.07 ± 0.16                    |
| NZ                              | 6.15 ± 0.33                    | 6.01 ± 0.33                    | 6.47 ± 0.33                    |

*Conductance is expressed as ml/min per 100 g per mm Hg. †Mean arterial pressure (MAP) is expressed as mm Hg. ‡p < 0.01 vs. control group. Ischemic zone (IZ) and normal zone (NZ) coronary conductance and the IZ/NZ ratio on day 33 in control dogs and dogs that received one and two doses of intracoronary basic fibroblast growth factor (bFGF).
Route of administration is an important determinant of response. These investigations provide evidence that the location of bFGF administration is a critical determinant of bFGF’s biologic activity, consistent with our previous observation that myocardial bFGF levels are determined principally by the proximity of injection (15). When iodine-125–labeled bFGF was injected into the left anterior descending coronary artery of dogs, bFGF activity in the territory of the injected artery was approximately sevenfold higher than that of the myocardium of the un.injected, control area (15). This concept is consistent with our observations across numerous investigations: bFGF has promoted collateral development when administered by the IC route (in the present IC substudy, as well as in a previous study [1]) and by the systemic arterial (LA) route (4,5); however, no effect was observed using the peripheral or central venous routes. The pericardial substudy represents the single exception to this principle, for the possible reasons discussed earlier. In contrast, bFGF toxicity appears to be largely route-independent, related to cumulative dose and/or duration of exposure, although the pericardial route is again an exception.

Study limitations and implications for clinical trials. Previously, we demonstrated the importance of ischemia or a transcollateral pressure gradient, or both, as primers for bFGF-induced collateral expansion (9). Basic FGF administration enhanced collateral perfusion when synchronized with the period of progressive coronary occlusion, but not when administered six months later. The present set of investigations provides evidence that proximity of injection is likewise a critical determinant of bFGF’s biologic activity. Thus, taken together, these studies suggest that optimal treatment would involve IC bFGF delivery during periods of myocardial ischemia, a matter of considerable practical importance for the design of clinical trials.

We have confirmed the ability of bFGF to promote collateral development when administered to healthy dogs subjected to progressive, single coronary artery occlusion whose coronary arteries are otherwise normal. These animals undergo a natural program of collateral development and appear to be responsive to the effects of IC bFGF. At the other end of the spectrum are dogs with mature collateral vessels—these animals are unresponsive to bFGF administration (9). It is possible that patients with chronic stable angina lie somewhere between these extremes, manifesting intermittent ischemia that could provide opportunities for bFGF responsiveness.

Conclusions. We have established proof of concept of bFGF-induced myocardial coronary collateral expansion using a clinically feasible IC route of bFGF administration; however, it is not known to what extent the results of these experiments can be extrapolated to the complex milieu of human ischemic heart disease. This is a question that can only be addressed through clinical trials.

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