Basic Fibroblast Growth Factor in Patients With Intermittent Claudication: Results of a Phase I Trial

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Bethesda, Maryland

OBJECTIVES
This phase I study was designed to evaluate the safety, tolerability and pharmacokinetics of intra-arterial basic fibroblast growth factor (bFGF) in patients with atherosclerotic peripheral arterial disease (PVD) and intermittent claudication. We also assessed the effects of basic fibroblast growth factor (bFGF) on calf blood flow as a measure of biologic activity.

BACKGROUND
Preclinical studies have shown that bFGF, an angiogenic peptide, promotes collateral development in animal models of myocardial and hind limb ischemia. The safety and efficacy of bFGF in patients is unknown, and early clinical trials are underway in coronary and peripheral arterial disease.

METHODS
A double-blind, placebo-controlled, dose-escalation trial was conducted in patients with claudication demonstrating ankle/brachial index \( \leq 0.8 \). Patients were randomly assigned to placebo (n = 6), 10 \( \mu g/kg \) of bFGF (n = 4), 30 \( \mu g/kg \) of bFGF once (n = 5) and 30 \( \mu g/kg \) of bFGF on two consecutive days (n = 4). Study drug was infused into the femoral artery of the ischemic leg. Detailed safety information including retinal photography for neovascularization were obtained through one year. Calf blood flow was measured with strain gauge plethysmography in the two higher dose treatment groups and in four placebo patients at baseline, one month and three to seven months after treatment.

RESULTS
Intra-arterial bFGF was safe and well-tolerated. The half-life was 46 \( \pm 21 \) min. Calf blood flow increased at one month by 66 \( \pm 26\% \) (mean \( \pm \) SEM) and at six months by 153 \( \pm 51\% \) in bFGF-treated patients (n = 9, p = 0.002). Flow did not change significantly in the placebo group.

CONCLUSIONS
In this initial randomized, double-blind, placebo-controlled trial in patients with atherosclerotic PVD and claudication, bFGF was well-tolerated. The data suggest a salutary biologic effect, and initiation of phase 2 trials is warranted. (J Am Coll Cardiol 2000;36:1239–44) © 2000 by the American College of Cardiology

Peripheral arterial disease (PVD) is a significant public health problem (1) for which pharmacologic therapies have not been consistently effective (2). In older adults, a strong association exists between intermittent claudication and subsequent mortality and cardiovascular morbidity (3–5).

Recently, significant research has focused on developing angiogenic therapies to provide novel approaches to the treatment of myocardial and limb ischemia. We and other investigators have demonstrated the potential of basic fibroblast growth factor (bFGF) to improve collateral development in animal models of myocardial (6–9) and hind limb (10–12) ischemia. On the basis of these findings, we initiated a phase I trial to evaluate the safety, tolerability and pharmacokinetics of intra-arterial bFGF in patients with atherosclerotic PVD and intermittent claudication. We also assessed the effects of bFGF on calf blood flow as a measure of biologic activity.

METHODS
Patient population. Selection criteria included age >40 years, a history of intermittent claudication for >6 months and ankle brachial index (ABI) <0.8 at rest or after 5 min of standardized exercise (13) (2 miles per h at a grade of 10°).

Exclusion criteria included gangrene, osteomyelitis, vascular interventions within three months of enrollment, serum creatinine >2.0 mg/dL, significant proteinuria, pre-existing or suspected malignancy and severe nonproliferative or proliferative diabetic retinopathy.

The study was performed at the National Heart, Lung, and Blood Institute, Bethesda, Maryland, and was approved by the Institutional Review Board. An independent Data Safety Monitoring Board monitored the safety of the drug. Nineteen patients met the total study criteria and were randomized after giving written informed consent.

Study protocol. At enrollment, a medical history and physical examination were conducted. Clinical laboratory studies were performed at screening (complete blood count,
These measurements were carried out in the two high-dose possible effects of bFGF on lower extremity blood flow. One patient randomized to bFGF 30 mg/kg group and over 15 min in the 30 mg/kg group, we amended the study protocol to assess bFGF groups using strain gauge plethysmography (Hokanson, Issaquah, Washington), as previously described (14). Measurements were made immediately before treatment, at 1 month and at 3 to 7 months (median 6 months) after treatment by an observer blinded to group assignment.

**Statistical analysis.** Data are expressed as mean ± SEM unless stated otherwise. For the analysis of pre- versus posttreatment differences, Friedman repeated measures analysis of variance on ranks was used. A p value <0.05 was considered to indicate statistical significance. Correction for pairwise multiple comparisons was made using the Student-Newman-Keuls method.

**RESULTS**

**Patients.** Baseline patient characteristics are summarized in Table 1. There were a number of imbalances in baseline characteristics, typical of a study of limited sample size.

**Safety.** The first subject randomized to bFGF received a 10 μg/kg bolus over 2 min. Systolic blood pressure decreased by 22% at 1 min and by 47% at 20 min, with onset of recovery at 25 min and return to baseline by 60 min, aided by infusion of saline. The patient remained asymptomatic. For subsequent subjects, the protocol was modified to deliver the study agent using an infusion pump over 4 min in the 10 μg/kg group and over 15 min in the 30 μg/kg group, and significant hypotension was not observed.

Retinal neovascularization was not detected during the course of the study. Mild transient proteinuria occurred in both placebo and bFGF-treated subjects, but there were no significant differences between groups in any hematological or biochemical tests. One patient age 74 with borderline thrombocytopenia (decrease in platelet count from 150,000 to 120,000), and follow-up data from this patient are included with the 30 μg/kg ×1 group.

Patients were hydrated with 500 mL of normal saline before study drug administration, and hydration was maintained at 60 to 75 mL per hour for 4 h after drug infusion. The femoral artery of the more ischemic leg, as determined by ABI, was cannulated percutaneously with an 18 gauge catheter (Arrow International, Inc. Reading, Pennsylvania), and the study agent was administered by intra-arterial infusion over 15 min. Heparin was not used. Hemodynamic monitoring was performed for 30 min after infusion. All patients underwent continuous Holter monitoring from the time of initial drug infusion to 36 h after the second treatment.

Venous blood samples were assayed for human bFGF at 0, 1, 3, 5, 10, 20, 30, 60, 120 and 180 min using a solid-phase ELISA kit (catalog No.DFB00, R&D Systems, Inc., Minneapolis, Minnesota) by Burleson Research Technologies, Inc., Raleigh, North Carolina.

**Measurement of calf blood flow.** After completion of the 1 month safety follow-up in the first block of patients (10 μg/kg bFGF), we amended the study protocol to assess possible effects of bFGF on lower extremity blood flow. These measurements were carried out in the two high-dose

<table>
<thead>
<tr>
<th><strong>Parameters</strong></th>
<th>bFGF-treated</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subjects</td>
<td>13</td>
<td>6</td>
</tr>
<tr>
<td>Age, years</td>
<td>68 ± 10</td>
<td>77 ± 9</td>
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<tr>
<td>Men</td>
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<td>Diabetes</td>
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<td>3</td>
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<td>Smokers</td>
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<tr>
<td>Hypertension</td>
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<tr>
<td>Previous vascular surgery</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Previous lower limb PTA</td>
<td>4</td>
<td>3</td>
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<tr>
<td>H/o CAD/CABG</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Cholesterol (mg/dL)</td>
<td>215 ± 55</td>
<td>181 ± 19</td>
</tr>
<tr>
<td>Baseline ABI</td>
<td>0.60 ± 0.28</td>
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</table>

Values are means ± SD.

ABI = ankle/brachial systolic blood pressure ratio; bFGF = basic fibroblast growth factor; CAD = coronary artery bypass grafting; CABG = coronary artery disease; H/o = history of; PTA = percutaneous transluminal angioplasty.

To convert values for cholesterol to mmol/L, multiply by 0.0259.

blood chemical analyses, urinalysis, 24-h urine for total protein and creatinine clearance) and repeated three days and one, two and four weeks after treatment. Screening tests for occult malignancy included chest X-ray, prostate specific antigen in men, mammogram, pelvic examination/pap smear in women and stool for occult blood. Comprehensive ophthalmologic examination, including retinal photography of the seven standard stereoscopic fields, was performed at baseline, one, three and 12 months. Patients answered a questionnaire with a review of systems, which included grading the degree of claudication as being the same, better or worse than usual. Exercise testing for measurement of ABI was performed using a treadmill speed of 2 miles per h at a 10° grade. Patients were required to be on a stable medication regimen six weeks before entry into the study and during the follow-up period.

Basic fibroblast growth factor was provided by Scios, Inc., Mountainview, California. Nineteen subjects were randomly assigned to bFGF, 10 μg/kg injected once, 30 μg/kg injected once or 30 μg/kg injected twice (on consecutive days). Randomization was in blocks of six, with two and four subjects assigned to placebo and bFGF, respectively. One patient randomized to bFGF ×2 doses did not receive the second dose, because of thrombocytopenia (decrease in platelet count from 150,000 to 120,000), and follow-up data from this patient are included with the 30 μg/kg ×1 group.

Patients were hydrated with 500 mL of normal saline before study drug administration, and hydration was maintained at 60 to 75 mL per hour for 4 h after drug infusion. The femoral artery of the more ischemic leg, as determined by ABI, was cannulated percutaneously with an 18 gauge catheter (Arrow International, Inc. Reading, Pennsylvania), and the study agent was administered by intra-arterial infusion over 15 min. Heparin was not used. Hemodynamic monitoring was performed for 30 min after infusion. All patients underwent continuous Holter monitoring from the time of initial drug infusion to 36 h after the second treatment.

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### Abbreviations and Acronyms

- **ABI** = ankle/brachial index or systolic blood pressure ratio
- **bFGF** = basic fibroblast growth factor
- **PVD** = peripheral arterial disease
- **VEGF** = vascular endothelial growth factor

**Table 1.** Characteristics of bFGF Treated and Placebo Patients: Baseline Demographics and Disease Status

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To convert values for cholesterol to mmol/L, multiply by 0.0259.
Pharmacokinetics of bFGF. Serum bFGF levels peaked at 1 min after infusion. Basic fibroblast growth factor had a rapid distribution phase with a volume of distribution of 0.66 ± 0.25 L/kg and an elimination half-life of 46 ± 21 min. In patients receiving two doses, baseline serum levels of bFGF were higher before the second dose of bFGF compared with day 1 (Table 2). The area of distribution under the curve demonstrated a dose response relationship (Fig. 1).

Calf blood flow. Placebo patients. The absolute changes in flow are shown in Table 3. One placebo patient had marked improvement in flow at 1 month (Fig. 2), with decrease below baseline at seven months (1.93, 3.43 and 1.54 mL/min/dL, respectively). A second placebo patient, who was hypertensive and diabetic, had a decrease in systolic blood pressure by 23 mm Hg between the first and second flow measurements and changes in medications that were made for clinical reasons before the final measurement. This patient’s flow at baseline, one month and five months was 0.43, 2.05 and 1.54 mL/min/dL. Despite these heterogeneous responses at 1 month, calf blood flow in the placebo group did not change significantly between baseline and six months (p = 0.27).

bFGF-treated patients. Resting calf blood flow improved significantly in bFGF-treated patients (Table 3).

The mean percent change in calf blood flow and vascular resistance measured at one and six months in bFGF-treated patients is shown in Figure 3. Combining the one- and two-dose groups, the nine bFGF-treated patients had a 66 ± 26% increase in calf blood flow at one month and a further significant increase of 153 ± 51% at six months (p < 0.05, one vs. six months); vascular resistance decreased by 39 ± 7% and 54 ± 9% at one and six months (p < 0.0001).

Symptoms. Of the four placebo-treated patients, one reported marked improvement in claudication and walking distances at one month (blood flow in the treated leg improved as well, Fig. 2), with worsening to baseline at seven months. Three of the five patients in the one-dose bFGF group, and three of the four patients in the two-dose bFGF group, reported marked symptomatic improvement at one month. No patient reported worsening of symptoms.

DISCUSSION

Previous studies of angiogenic factors and significance of this study. There is a significant body of animal data demonstrating proof of concept of salutary effect of peptide growth factors in hind limb and myocardial ischemia, and multiple phase 1 and early phase II trials are in progress. Recent phase 1 studies have suggested tolerability of vascular endothelial growth factor (VEGF) gene transfer (15–17), intracorony VEGF protein (18), intramyocardial acidic FGF protein (19) and intracoronary (20) and perivascular (21) bFGF. Gene transfer of plasmid DNA encoding VEGF has shown favorable results in patients with critical limb ischemia in an uncontrolled open-label study (22). To our knowledge, this is the first report of a randomized, double-blind, placebo-controlled study of bFGF administration in humans with peripheral vascular disease. We have shown that intra-arterial bFGF is well-tolerated and free of significant adverse effects in subjects with intermittent claudication.

Pharmacokinetics of bFGF. We found that the acute hypotensive effects of bFGF can be mitigated by slowing the rate of infusion. The serum half-life was 46 min, in keeping with our preclinical studies (7). Basic fibroblast growth factor had a small volume of distribution, indicating that it is not extensively tissue-bound. However, in the group receiving two doses, the level of bFGF was higher before the

<table>
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<th>bFGF Dose/Regimen</th>
<th>10 µg/kg</th>
<th>30 µg/kg</th>
<th>First Dose</th>
<th>Second Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>baseline (bFGF) (pg/mL)</td>
<td>32 ± 19</td>
<td>78 ± 39</td>
<td>&lt;10</td>
<td>151 ± 16</td>
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<tr>
<td>peak (bFGF) (ng/mL)</td>
<td>29.1 ± 1.2</td>
<td>57 ± 9.5</td>
<td>47.2 ± 9.3</td>
<td>61.5 ± 20.6</td>
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<tr>
<td>AUC (ng* min/mL)</td>
<td>634 ± 104</td>
<td>1325 ± 594</td>
<td>3560 ± 511</td>
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</tr>
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<td>t 1/2 (min)</td>
<td>32 ± 3</td>
<td>42 ± 9</td>
<td>73 ± 23</td>
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Serum levels of bFGF were quantified by ELISA at 1, 3, 5, 10, 20, 30, 60, 120 and 180 min after infusion. Serum bFGF levels peaked at 1 min after infusion. Basic fibroblast growth factor had a rapid distribution phase with a volume of distribution of 0.66 ± 0.25 L/kg and an elimination half-life of 46 ± 21 min. AUC = the area under the concentration-time curve; bFGF = basic fibroblast growth factor.

![Figure 1](image-url)
second dose compared with baseline, suggesting the presence of a reservoir for bFGF, likely the vascular subendothelial matrix (23), with slow release of the peptide into the circulation over time.

**Potential toxicity of bFGF.** Basic fibroblast growth factor has the potential to induce a nephropathy with proteinuria at higher doses or with repeated administration over time (24). We were able to show that two doses of 30 μg/kg of bFGF did not have appreciable effects on proteinuria or serum creatinine levels in our patients. Although diabetics were included, larger numbers of patients are needed before a deleterious effect on renal function can be safely ruled out in diabetics and in patients with proteinuria exceeding 200 mg/24 h. Endothelial growth factors have been implicated as mediators of intraocular neovascularization (25,26). In our study of patients with normal retinae at baseline and in individuals with mild to moderate nonproliferative retinopathy (three patients enrolled in the study had nonproliferative retinopathy), bFGF did not induce new vessel formation. The effects of bFGF on hypoxic or ischemic retinae or on diabetic proliferative retinopathy remain unknown. Thrombocytopenia that occurred in a bFGF-treated patient was transient and asymptomatic, consistent with preclinical safety data with bFGF (7,8).

**Calf blood flow changes with bFGF.** This was a phase I study not primarily designed to assess efficacy. Calf blood flow was measured as an exploratory secondary end point. We found that one and two intra-arterial doses of bFGF at 30 μg/kg improved calf blood flow in the treated leg. This was accompanied by subjective improvement in symptoms in a majority of treated patients, consistent with a biologic effect. There appears to be a dose-response relationship between the dose of bFGF and calf blood flow. However, the differences between the two doses were not statistically significant, a fact probably due to the small numbers of patients studied. Calf blood flow in the treated patients continued to improve three to seven months after treatment, suggesting that activities of daily living may have provided an ischemic milieu that sustained and improved the angiogenic effect of bFGF with time. The acute vasodilatory effects of bFGF could have contributed to collateral development (27); however, other vasodilators have failed to produce consistent effects on collateral growth (28,29).

These results on calf blood flow are preliminary and cannot be considered definitive; however, the results are clinically encouraging and warrant further study.

**Placebo component in angiogenic trials.** A large placebo response is common in claudication trials. In a review of 75

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**Table 3.** Resting Calf Blood Flow (ml/min/dl)

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>1 Month</th>
<th>6 Months</th>
<th>p (Baseline vs. 6 Months)</th>
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</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>1.29 ± 0.33</td>
<td>2.15 ± 0.44</td>
<td>1.35 ± 0.17</td>
<td>0.27</td>
</tr>
<tr>
<td>30 μg/kg × 1</td>
<td>1.11 ± 0.21</td>
<td>1.44 ± 0.23</td>
<td>2.09 ± 0.55</td>
<td>0.046</td>
</tr>
<tr>
<td>30 μg/kg × 2</td>
<td>0.60 ± 0.10</td>
<td>1.10 ± 0.09</td>
<td>1.91 ± 0.06</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Flow improved significantly between 1 and 6 months in bFGF-treated patients.

bFGF = basic fibroblast growth factor.

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**Figure 2.** Resting calf blood flow in all placebo and bFGF-treated patients at baseline and 1 month. The greatest improvement in calf blood flow occurred in a placebo patient. bFGF = basic fibroblast growth factor.
studies, 84% of open-label or uncontrolled studies suggested benefit from drug treatment, whereas only 32% of placebo-controlled trials reported improvement (2). Our study illustrates the heterogeneity of responses in this patient population. Indeed, the patient manifesting the greatest improvement in calf blood flow in our study was a placebo patient (Fig. 2). Recently, the placebo response was well-illustrated in coronary artery disease (CAD) patients receiving intravascular VEGF (18). Several phase I/II angiogenic trials are being planned or are under way in CAD and PVD patients, and the need for placebo-controlled studies is evident.

The best approach to angiogenic drug delivery (recombinant protein vs. gene therapy) is unclear, and the long-term safety of these agents is unknown.

Conclusions. Our phase I study indicates that intrarterial bFGF is feasible and well-tolerated in PVD patients and free of significant adverse effects at short-term follow-up. The preliminary findings of a salutary effect on calf blood flow require confirmation in a larger trial sufficiently powered to determine efficacy.

Acknowledgments
We are indebted to Frances Loscalzo for assisting in the study, William Schenke for graphics, Stephen C. Piscitelli for pharmacokinetics of bFGF, Karl G. Csaky for ophthalmological examinations, Dr. Felton Anderson for patient referrals and Scios, Inc. for supplying bFGF.

Figure 3. Mean percent change in resting calf blood flow and vascular resistance in five patients who received one dose of 30 μg/kg of bFGF intraarterially, four patients who received two doses of 30 μg/kg of bFGF and all nine treated patients. Values represent mean ± SEM. The difference between time points (baseline, 1 and 6 months) is tested with analysis of variance. bFGF = basic fibroblast growth factor.

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