Therapeutic Angiogenesis With Recombinant Fibroblast Growth Factor-2 Improves Stress and Rest Myocardial Perfusion Abnormalities in Patients With Severe Symptomatic Chronic Coronary Artery Disease

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Background—We report the effects of the administration of recombinant fibroblast growth factor-2 (rFGF-2) protein on myocardial perfusion using single photon emission computed tomography imaging in humans with advanced coronary disease.

Methods and Results—A total of 59 patients with coronary disease that was not amenable to mechanical revascularization underwent intracoronary (n=45) or intravenous (n=14) administration of rFGF-2 in ascending doses. Changes in perfusion were evaluated at baseline and again at 29, 57, and 180 days after rFGF-2 administration. In this uncontrolled study, perfusion scans were analyzed by 2 observers who were blinded to patient identity and test sequence; scans were displayed in random order, with scans from nonstudy patients randomly interspersed to enhance blinding. Combining all dose groups, a reduction occurred in the per-segment reversibility score (reflecting the magnitude of inducible ischemia) from 1.7±0.4 at baseline to 1.1±0.6 at day 29 (P<0.001), 1.2±0.7 at day 57 (P<0.001), and 1.1±0.7 at day 180 (P<0.001). The 37 patients with evidence of resting hypoperfusion had evidence of improved resting perfusion: their per-segment rest perfusion score of 1.5±0.5 at baseline decreased to 1.0±0.8 at day 29 (P<0.001), 1.0±0.8 at day 57 (P=0.003), and 1.1±0.9 at day 180 (P=0.11).

Conclusions—These preliminary data suggest that the administration of rFGF-2 to patients with advanced coronary disease resulted in an attenuation of stress-induced ischemia and an improvement in resting myocardial perfusion; these findings are consistent with a favorable effect of therapeutic angiogenesis. (Circulation. 2000;102:1605-1610.)

Key Words: angiogenesis ▪ heart diseases ▪ growth substances

For patients with advanced symptomatic coronary artery disease that is not amenable to standard mechanical revascularization strategies, numerous innovative approaches are being developed. These approaches include promoting the growth of new blood vessels in the myocardium using several potential compounds, delivery vectors, and delivery mechanisms to the ischemic myocardium.1–4

Basic fibroblast growth factor (bFGF) is a 16-kDa peptide and a pluripotent mitogen.3,4 In numerous animal models, it has reportedly promoted angiogenesis, improved myocardial perfusion,5–9 and acutely improved endothelial vasodilatory function.10 In the present study, we report the impact of the administration of recombinant fibroblast growth factor-2 (rFGF-2) on stress and rest myocardial perfusion using gated single-photon emission computed tomography (SPECT) myocardial perfusion imaging in a phase 1 trial in humans with advanced symptomatic coronary artery disease.

Methods

Patient Population

Patients were eligible for inclusion in this protocol if they had advanced chronic coronary disease, were suboptimal candidates for percutaneous transluminal coronary angioplasty (PTCA) or coronary
artery bypass grafting (CABG), and had clinical evidence of inducible ischemia, as defined by the presence of exertional angina, ischemic ECG changes on stress testing, or evidence of a reversible perfusion defect on perfusion imaging.10 Exclusion criteria included the presence of decompensated heart failure and/or severe left ventricular dysfunction (ejection fraction <20%); recent myocardial infarction (<3 months from study start); new onset or episode of unstable angina within the previous 3 weeks; recent PTCA, CABG, or cerebral vascular event (within 6 months); evidence of proliferative retinopathy; creatinine clearance <80 mL/min; or any recorded malignancy within the past 10 years (except for curatively treated non-melanoma skin cancer). Patients with a history of diabetes were eligible if they had no evidence of proliferative retinopathy or severe nonproliferative retinopathy by ophthalmological examination and if they had no evidence of microalbuminuria.

The study was conducted at 2 institutions (Beth Israel-Deaconess Medical Center, Boston, Mass and Emory University Hospital, Atlanta, Ga). The protocol and consent form were approved by the Human Investigation Committee at both institutions.

Study Procedures and Administration of rFGF-2
After providing informed consent, patients underwent baseline testing and coronary angiography. If the coronary anatomy remained suboptimal for PTCA or CABG, rFGF-2 was infused via a Baxter infusion pump through standard diagnostic catheters into 2 major myocardial territories via patent native coronary arteries or patent bypass grafts, after ensuring the patient had a mean pulmonary capillary wedge pressure >12 mm Hg. Heparin (40 U/kg) was administered intravenously at least 10 minutes before rFGF-2 administration. The study drug was infused by a pump over 20 minutes (10 minutes per vessel).

rFGF-2 (Chiron Technologies) was infused at one of multiple doses from 0.33 µg/kg (ideal body weight) up to 48 µg/kg. Because this was an integrated dose-escalation study of safety and tolerability, the initial group of patients received the lowest dose and, as tolerance was demonstrated, subsequent groups of patients received successively higher doses. At least 4 subjects were studied at each dose. If no subject within a specific dosing level experienced dose-limiting toxicity (as defined by the protocol) within 6 days, the dose was escalated for the next group; if one subject within a dose group experienced dose-limiting toxicity, an additional 4 subjects were studied at that dose before higher doses were studied. Additional patients received an intravenous infusion of rFGF-2 in doses of 18 or 36 µg/kg ideal body weight.

Stress Testing Protocol
Stress testing was performed before the administration of rFGF-2 and then repeated ~29, 57, and 180 days after administration. For patients who were able to exercise, symptom-limited treadmill exercise was performed under standard supervision and continuous ECG monitoring. For patients not able to perform treadmill exercise, a standard pharmacological vasodilator stress test with intravenous dipyridamole was performed (n = 5).

Gated SPECT Myocardial Perfusion Imaging
To analyze myocardial perfusion, a dual-isotope gated SPECT thallium 201/technetium-99m sestamibi protocol was used. For the resting studies, 3 to 4 mCi of thallium 201 was injected, with SPECT acquisition beginning 10 to 20 minutes after injection. Images were acquired with an ADAC Vertex camera system at 72 and 161 keV energy peaks, with a 30% and 20% window, respectively.

For the stress perfusion studies, 22 to 32 mCi of technetium-99m sestamibi was injected 1 to 2 minutes before the completion of treadmill exercise or at 2 to 4 minutes after completion of the infusion of dipyridamole. Sestamibi stress SPECT images were acquired beginning 15 to 45 minutes after the completion of treadmill stress and ~45 to 90 minutes after the infusion of dipyridamole. Acquisition was performed using an energy peak of 140 keV, with a 20% window, a 64 × 64 matrix, and a low-energy, high-resolution collimator. The stress sestamibi images were acquired in gated SPECT mode for simultaneous analysis of perfusion and function. Images were reconstructed using standard software on the ADAC system. The gated SPECT images were analyzed using QGS software, which was previously validated against numerous other quantitative techniques11 for the derivation of ejection fraction.

SPECT Image Analysis
Because this was an open-label study, efforts were made to ensure the readers were blinded. First, images were blinded with respect to patient identity and timing (baseline or follow-up studies). These blinded images were then displayed individually and in completely random order. The images were interpreted by a consensus of the 2 observers, who divided the total number of patient images into 4 separate reading sessions, which were completed over a period of 6 weeks. Finally, to further blind the analysis, similarly acquired images from patients with coronary artery disease who were not participating or not eligible for the study were also interspersed randomly throughout the image reading sequence, with the readers also blinded to these scans.

The images were analyzed using a widely validated semiquantitative grading system in a 20-segment left ventricular model.12 Each segment was graded on a 5 point scale, as follows: 0, normal; 1, slightly reduced; 2, mildly reduced; 3, moderately reduced; and 4, severely reduced (absent) activity. A segment was determined to have an ischemic defect if the assigned grade at stress decreased or normalized on the rest images (reversibility score [stress score − rest score] was ≥1); a segment had a fixed defect if the abnormal grade at stress remained the same on rest imaging. Fixed defects were then subgrouped on the basis of the severity into mild-to-moderate (scores 1, 2, and 3) and very severe (score of 4) groups. The global extent of the perfusion abnormality and ischemia were assessed by summing the individual scores from the 20 segments,12 and they were expressed as the summed stress and summed reversibility scores, respectively.

Statistical Analysis
To account for the variable number of abnormal segments per patient in each analysis of rFGF-2 effect, an average score per abnormal segment was calculated for each patient by dividing the summed scores among the abnormal segments for an individual patient by the number of abnormal segments. This was repeated for each time point, and the differences between the patient-specific averages were calculated. Data are expressed as mean ± SD. The averaged differences were compared using a modified paired t test and weighted according to the number of analyzed segments per patient, thereby adjusting for the heterogeneity of variances resulting from the differing number of abnormal segments per patient. All reported probability values are 2-sided, and values <0.05 are considered statistically significant.

Results
Patient Population
Of the 59 patients who met all inclusion criteria and no exclusion criteria, 45 received an intracoronary infusion of rFGF-2 and 14 received an intravenous infusion of rFGF-2. All had baseline and at least 1 follow-up study of myocardial perfusion. Their mean age was 60.1 ± 9.8 years, 95% were male, 81% had a history of prior CABG (24% had >1 CABG procedure), 49% had a history of prior PTCA, and 44% had a history of myocardial infarction. Patients were generally functionally impaired, with 51%, 20%, and 5% being in New York Heart Association functional classes 2, 3, and 4, respectively.

Effect of rFGF-2 on Inducible Ischemia
No consistent pattern of improvement existed in scores representing global stress perfusion or inducible ischemia.
Among all patients, summed stress scores were 11.9 ± 6.8 at baseline, 10.6 ± 6.8 at day 29, 12.2 ± 9.9 at day 57, and 12.8 ± 6.9 at day 180 (all $P$ > 0.05 NS). Similarly, summed reversibility scores (reflecting the global magnitude of ischemia) were 8.3 ± 7.0 at baseline, 7.3 ± 6.2 at day 29, 8.3 ± 7.0 at day 57, and 7.9 ± 6.1 at day 180 (all $P$ > 0.05 NS). Among the 51 patients with evidence of inducible ischemia at baseline, however, global scores at certain time points were improved. Summed stress scores in this group were 13.8 ± 8.3 at baseline, 12.3 ± 8.5 at day 29 ($P$ = 0.03), 14.2 ± 9.6 at day 57 ($P$ = NS), and 14.7 ± 8.7 at day 180 ($P$ = NS); their summed reversibility scores were 9.8 ± 6.5 at baseline, 8.3 ± 6.0 at day 29 ($P$ = 0.007), 9.7 ± 6.9 at day 57 ($P$ = NS), and 9.0 ± 6.1 at day 180 ($P$ = 0.025).

Because we expected the effect of rFGF-2 administration to be most demonstrable in segments with baseline ischemia, we analyzed changes in perfusion in those segments specifically. Among the 59 patients, 51 demonstrated scintigraphic evidence of inducible ischemia at baseline in an average of 5.3 of the 20 segments. At baseline, the mean reversibility score per ischemic segment was 1.7 ± 0.4 (corresponding, on average, to a moderately severe reversible defect of the ischemic left ventricular segments). There was a consistent and sustained reduction of the extent and severity of inducible ischemia (Figures 1 and 2): the per-segment reversibility score decreased to 1.1 ± 0.6 at day 29 ($P$ < 0.001), 1.2 ± 0.7 at day 57 ($P$ < 0.001), and 1.1 ± 0.7 at day 180 ($P$ < 0.001). A directionally similar reduction in the extent of inducible ischemia was seen in all dose groups at all time points, although not all reached statistical significance because the number of patients within the individual dose groups was relatively small. At each time point, a substantial proportion (≥ 40%) of segments with an improved reversibility score had improved by > 1 grade of severity (Figure 3).

Because the reversibility score is derived directly from the stress and the rest scores, it is conceivable that a reduction in the reversibility score may result either from a reduction in inducible ischemia or, alternatively, from an increase in the size of the resting perfusion defect, indicating more extensive infarction on serial follow-up. However, no significant change occurred in the rest score in the ischemic segments at any time point. Thus, the reduction in the reversibility score was entirely due to a reduction in the stress score in the ischemic segments, which is consistent with the concept that the administration of rFGF-2 resulted in a reduction in the magnitude of inducible ischemia: the per-segment stress score in the ischemic segments was reduced from 2.1 ± 0.5 at baseline to 1.6 ± 0.7 at day 29 ($P$ < 0.001), 1.6 ± 0.8 at day 57 ($P$ < 0.001), and 1.7 ± 0.8 at day 180 ($P$ < 0.001).

In contrast to the results in segments with ischemic defects at baseline, segments with fixed defects of any magnitude at baseline had no significant change in stress perfusion scores across the course of the trial.
Changes in Rate-Pressure Product and Background Medications

Because the magnitude of myocardial ischemia is related to oxygen demand, serial changes in the rate-pressure product were examined to assess whether the diminution in ischemia across the trial may have merely been related to diminution in demand. At baseline, among the 51 patients with inducible ischemia, the rate-pressure product was 18 787±6709 mm Hg×bpm. A small but significant decrease in the rate-pressure product occurred among patients undergoing the day 29 stress test (to 16 499±5276 mm Hg×bpm; P=0.03), no change occurred compared with baseline at day 57 (17 933±6563 mm Hg×bpm; P=0.25), and an increase occurred at day 180 (22 113±6463 mm Hg×bpm; P<0.001). The extent of inducible ischemia at these time points remained significantly diminished compared with baseline. Hence, it is unlikely that the observed diminution in inducible ischemia was solely due to diminished oxygen demand.

Of the 59 patients, only 5 had recorded medication changes from their background medications at baseline. One patient had a decrease in diltiazem (420 mg to 240 mg QD) and an increase in isosorbide mononitrate (from 40 mg to 60 mg QD) just after the baseline study. Two patients had a decrease (by 50%) in their isosorbide mononitrate dosing, one before the first follow-up study and one after the day 57 study. Two patients had a medication added; one added metoprolol (25 mg QD) and the other added amlodipine (5 mg QD). Both medications were added just after the baseline study. Thus, there was no consistent increase of dosing or new medications added in enough of a proportion of the population to suggest that concomitant medications played an important role in the reported results.

Effect of rFGF-2 Administration on Resting Myocardial Perfusion

To evaluate the potential impact of rFGF-2 administration on resting myocardial perfusion, patients with a resting thallium 201 perfusion abnormality of mild-to-moderate severity (scores of 1, 2, or 3) were analyzed, because previous studies demonstrated that segments with very severe rest thallium 201 perfusion abnormalities are unlikely to contain clinically significant viable myocardium.14–17 In the 37 patients who had at least one segment with such a rest perfusion abnormality, an improvement in rest perfusion occurred: the per-segment rest score in the abnormal segments of 1.5±0.5 at baseline decreased to 1.0±0.8 at day 29 (P<0.001), 1.0±0.8 at day 57 (P=0.003), and 1.1±0.9 at day 180 (P=0.11). These patients also had a reduction in the stress score in these segments, suggesting an improvement in both stress and rest perfusion.

Effect of rFGF-2 Administration on Global Ejection Fraction

No important changes occurred in ejection fraction: it was 47±12% at baseline, 49±11% at day 29 (P=0.05), 48±11% at day 57 (P=NS), and 47±11% at day 180 (P=NS).

Discussion

The present data indicate that the administration of rFGF-2 to patients with symptomatic advanced coronary disease results in an attenuation of the magnitude of stress-induced myocardial ischemia, as well as an improvement in resting perfusion in territories with diminished myocardial perfusion at rest. The absence of a consistent reduction in oxygen demand during stress in these studies (as reflected by the peak heart rate–blood pressure product) suggests that the observed improvement in perfusion was predominantly related to improved myocardial blood supply and coronary flow reserve.

The potential for the application of therapeutic angiogenesis in humans is based on a foundation of information derived from animal models of chronic ischemia. These studies have demonstrated enhanced development of collaterals,9 increased maximum collateral flow to ischemic zones during pharmacological vasodilatation,26 evidence of angiogenesis and diminished vascular resistance after intrapericardial administration,18,25 an increased number of collaterals and capillaries within the ischemic zone,20 and enhanced regional function in a porcine model.21

Human studies evaluating the use of angiogenic factors to this point have generally involved small numbers of patients. Losordo and colleagues22 reported on 5 patients treated with direct intramyocardial injection of naked plasmid DNA encoding vascular endothelial growth factor (VEGF), and they predominantly reported a reduction in the resting perfusion abnormalities seen in dobutamine septamibi SPECT studies. Rosengart and coworkers,23 using direct myocardial injection of VEGF cDNA delivered via an adenovirus transfer vector, found no overall change in perfusion, although there was a slight reduction in the extent of ischemia in 4 of the 6 patients receiving VEGF as the sole therapy. Schumacher and colleagues24 reported on studies of 20 patients undergoing intraoperative application of FGF-1 into the territories of the left anterior descending coronary artery, which were also undergoing surgical revascularization. They found evidence of enhanced collateralization and apparent capillary proliferation in the anterior wall by angiographic contrast techniques in the treated patients compared with controls. In a preliminary analysis, Hendel et al25 described 15 patients undergoing intracoronary administration of VEGF protein; they found no change in global stress perfusion scores among the entire group, although an improvement in global resting perfusion score was observed. In a subgroup receiving a higher intracoronary VEGF dose, 5 of 7 patients had improved stress and resting perfusion scores. Laham et al26 studied 24 patients who, at the time of clinically indicated bypass surgery, underwent implantation of heparin-alginate beads, resulting in the local myocardial release of rFGF-2 in nonrevascularizable ischemic territories. The high-dose bFGF group demonstrated a reduction in stress defect size on septamibi imaging compared with the low-dose group and with a placebo control group.

The present data extend these previous observations by studying a larger cohort of patients and by indicating in this group a reduction in the magnitude of stress-induced ischemia, which seemed to be sustained over 6 months of follow-
up. This analysis was focused on regions of the myocardium with inducible ischemia or resting perfusion abnormalities at baseline, the territories most likely to demonstrate a response to angiogenic therapy. No consistent improvement occurred in global perfusion or ischemia scores, which is similar to the data reported by Hendel et al.\textsuperscript{25} using VEGF. These findings are consistent with the concept that few patients had complete resolution of inducible ischemia; rather, the changes in perfusion were modest in magnitude and/or limited in extent.

The exact mechanism and anatomic substrate by which the administration of rFGF-2 resulted in improved myocardial ischemia and resting blood flow cannot be determined with certainty from this data set. Schapers\textsuperscript{27} has pointed out that improved myocardial blood flow after the administration of angiogenic agents may take place via 2 mechanisms: either (1) through an enhanced capillary network resulting from the new budding of capillaries or (2) through recruitment, remodeling, and growth of preexisting muscular arterioles, which may be visualized as an epicardial collateral network during angiography. These mechanisms have been referred to as angiogenesis and arteriogenesis, respectively. SPECT radionuclide studies of myocardial perfusion at stress and rest, such as that used in this study, demarcate the delivery of nutritive perfusion and require the presence of viable myocardial cells for uptake, retention, and subsequent imaging of the tracer.\textsuperscript{28} Thus, one cannot infer that an improvement in flow and tracer uptake is due to a particular vessel type or size. In several previously reported studies examining the relation between the presence of angiographic collaterals and the extent and severity of myocardial ischemia by radionuclide perfusion studies, some studies seemed to show a protective effect of angiographically visible collaterals, with the presence of collaterals being associated with diminished evidence of inducible ischemia.\textsuperscript{29,30}

Enthusiasm about the current results must be tempered by the setting of the present study. This study was an uncontrolled phase I safety study, with the myocardial perfusion imaging data being analyzed as a secondary end point. All patients received the study drug in an open-label fashion. The SPECT radionuclide perfusion data reported herein, however, are perhaps more objective indicators of response in an uncontrolled trial, because they are not subject to a placebo effect, as are the clinical parameters reported by patients or exercise time. The possibility that the observed reduction in stress-induced ischemia was due to chance or random variation is lessened by the fact that changes were seen consistently on all follow-up studies, despite the fact that scans were read in completely random order with the observers blinded to the timing of the studies within the serial sequence of testing. In the setting of this uncontrolled open-label trial, extensive efforts were made to blind the experienced readers to the sequence of the scans, including incorporating scans of patients not participating in the trial. Nevertheless, confirmation of these results awaits the completion of larger, placebo-controlled trials, in which the effect of different levels of exercise is removed by performing serial pharmacological stress testing with SPECT perfusion imaging or imaging at matched exercise levels.

Hence, in patients with symptomatic advanced coronary artery disease, these preliminary data suggest that rFGF-2 attenuates the magnitude of stress-induced ischemia and improves resting myocardial blood flow among a subset of patients with resting hyperperfusion. The findings are consistent with a favorable but modest effect of therapeutic angiogenesis with this agent, resulting in improved myocardial blood supply and coronary flow reserve. Should these data be confirmed in upcoming and ongoing trials and if they are accompanied by improvements in clinical parameters, they may signal the beginning of an important new approach to patients with advanced symptomatic coronary artery disease: medical revascularization with agents promoting therapeutic angiogenesis.

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References


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