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A Randomized, Double-Blind, Placebo-Controlled Trial of Ad5FGF-4 Gene Therapy and its Effect on Myocardial Perfusion in Patients With Stable Angina

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OBJECTIVES The primary objective of this study was to determine whether intracoronary administration of the adenoviral gene for fibroblast growth factor (Ad5FGF-4) can improve myocardial perfusion compared with placebo.

BACKGROUND Animal studies and observational clinical studies have shown improvement in perfusion of the ischemic myocardium using genes encoding angiogenic growth factors; however, randomized, double-blind data in humans are lacking.

METHODS We performed a randomized, double-blind, placebo-controlled trial of intracoronary injection of 10^{10} adenoviral particles containing a gene encoding fibroblast growth factor (Ad5FGF-4) to determine the effect on myocardial perfusion. Fifty-two patients with stable angina and reversible ischemia comprising >9% of the left ventricle on adenosine single-photon emission computed tomography (SPECT) imaging were randomized to gene therapy (n = 35) or placebo (n = 17). Clinical follow-up was performed, and 51 (98%) patients underwent a second adenosine SPECT scan after 8 weeks.

RESULTS Overall (n = 52), the mean total perfusion defect size at baseline was 32.4% of the left ventricle, with 20% reversible ischemia and 12.5% scar. At eight weeks, Ad5FGF-4 injection resulted in a significant reduction of ischemic defect size (4.2% absolute, 21% relative; $p < 0.001$) and placebo-treated patients had no improvement ($p = 0.32$). Although the change in reversible perfusion defect size between Ad5FGF-4 and placebo was not significant (4.2% vs. 1.6%, $p = 0.14$), when a single outlier was excluded a significant difference was observed (4.2% vs. 0.8%, $p < 0.05$). Ad5FGF-4 was well tolerated and did not result in any permanent adverse sequelae.

CONCLUSIONS Intracoronary injection of Ad5FGF-4 showed an encouraging trend for improved myocardial perfusion; however, further studies of therapeutic angiogenesis with Ad5FGF-4 will be necessary. (J Am Coll Cardiol 2003;42:1339–47) © 2003 by the American College of Cardiology Foundation

Myocardial ischemia is a leading cause of death and disability in the developed world. Established treatment for myocardial ischemia has to date consisted of revascularization using either percutaneous or surgical techniques to improve blood supply to the myocardium, or pharmacotherapy designed to limit myocardial oxygen demand. Many patients prove to be ineligible or refractory to the conventional therapies, and percutaneous or surgical revascularization is associated with a distinct set of risks. Newer approaches to improve blood flow to the myocardium have focused on the biology of enhancing growth and the development of new blood vessels. Despite encouraging reports from uncontrolled pilot studies, two recent clinical trials using the angiogenic proteins vascular endothelial growth factor-165 and basic fibroblast growth factor (FGF)-2 have reported no significant difference in treadmill exercise time in patients with severe ischemic heart disease compared with placebo (1,2). These negative findings may be due to the short duration of effect of the protein, and may be overcome by administering the gene, which results in sustained production of the angiogenic protein. We have previously shown that intracoronary administration of the gene encoding FGF-4 delivered using an adenoviral vector increased treadmill exercise time by 1.5 min compared with 0.9 min in placebo-treated patients with stable angina (3). An increase of 1 min compared with placebo was observed

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Abbreviations and Acronyms

ANOVA = analysis of variance
CABG = coronary artery bypass graft
CK = creatine kinase
FGF = fibroblast growth factor
HIV = human immunodeficiency virus
RPDS = reversible perfusion defect size
SGOT = serum glutamic-oxaloacetic transaminase
SGPT = serum glutamic-pyruvic transaminase
SPECT = single-photon emission computed tomography
^{99m}Tc = technetium-99m

12 weeks after treatment using a dose of 10^{10} viral particles (3). Although exercise duration is of prognostic importance, it does not directly measure myocardial perfusion.

Nuclear scintigraphy using quantitative tomographic techniques is highly accurate and reproducible and is a reliable indicator of myocardial perfusion (4–7). Moreover, the size of an ischemic perfusion defect has been shown in several studies to be a predictor of the risk of adverse cardiac events over a period of several years (8–11). Therefore, reduction in ischemic perfusion defect size would provide evidence that the therapy has increased blood supply to the myocardium, and may ultimately improve prognosis. Accordingly, we undertook a randomized, double-blind, placebo-controlled trial to determine the effects of intracoronary administration of adenoviral delivered FGF-4 gene therapy (Ad5FGF-4) on myocardial perfusion measured as scintigraphic ischemic defect size in patients with coronary artery disease. We selected a dose of 10^{10} viral particles of Ad5FGF-4 based on the findings of Grines et al. (3).

METHODS

Objectives. The primary objective of this study was to determine whether intracoronary administration of 10^{10} viral particles of Ad5FGF-4 could significantly decrease the size of an adenosine-induced ischemic left ventricular perfusion defect size compared with placebo. An important additional aspect of the primary study objective was to evaluate the safety of Ad5FGF-4, including specific risks related to the virus or related to FGF-4.

Study design and population. The study was approved by the Food and Drug Administration, Recombinant DNA Advisory Committee, appropriate institutional review boards and biosafety committees, and was conducted in accordance with the International Conference on Harmonization and Good Clinical Practice Guidelines. All patients provided written informed consent. Patients age 30 to 75 years with stable angina (≥4 weeks, angina class 2 to 4) who were symptomatic despite using antianginal drugs and who were not optimal candidates for revascularization by surgical or percutaneous means were studied. Patients could have single or multivessel coronary artery disease provided at least one proximal major vessel (coronary artery or bypass graft) had <70% stenosis to allow adequate infusion of study product. Patients were included if left ventricular ejection fraction determined by gated technetium-99m (^{99m}Tc)-sestamibi single-photon emission computed tomography (SPECT) was ≥30% and adenosine-induced (ischemic) left ventricular reversible perfusion defect size (RPDS) was >9%.

Exclusion criteria were as follows: left main coronary arterial stenosis ≥70% (unless protected with a patent graft), bypass surgery within the past 6 months, angioplasty within 4 months, unstable angina, patients requiring immediate revascularization, myocardial infarction <6 weeks, chronic heart failure (class 4), chronic atrial fibrillation/flutter, human immunodeficiency virus (HIV) positive, chronic immunosuppressive therapy, hepatic disease, and previous administration of any gene product or growth factor protein. Other exclusions were type 1 diabetes, patients with severe pre-proliferative or proliferative retinopathy, malignant tumors within the past 10 years, thrombocytopenia ($<130 \times 10^3/\mu$l), use of abciximab within 30 days, patients of child-bearing potential, and patients in whom adenosine administration was contraindicated.

Screening. Baseline observations and tests included history, physical, and ophthalmic examination, ^{99m}Tc-sestamibi gated SPECT imaging at rest and after pharmacologic stress with adenosine, Duke Activity Status Index (administered by the same person for a given patient throughout the study), assessment of angina class, diary for noting angina attacks and nitroglycerin use, clinical hematology, blood chemistry, liver function tests, urinalysis, HIV, tests for hepatitis B and C, troponin T, creatine kinase (CK)-myoglobin fraction and CK (isoenzyme), serum FGF-4 level, tumor screening involving history, physical, rectal, and pelvic examination, Papanicolaou smear and mammogram (women), prostate-specific antigen (men), stool for occult blood, proctosigmoidoscopy or colonoscopy if indicated, and other tests as necessary. Specialist opinion was obtained to evaluate abnormal symptoms, signs, or tests suspicious of malignancy.

Gene product preparation and administration. The Ad5FGF-4 gene consists of a recombinant human adenovirus (serotype 5) made replication-deficient by the deletion of E1A/E1B genes that are replaced by the human FGF-4 gene, driven by a cytomegalovirus promoter.

After the completion of baseline observations and tests, patients fulfilling the entry criteria were randomized (in the ratio of two active to one placebo) to receive intracoronary Ad5FGF-4 or matching placebo (vehicle) in a double-blind manner. A coronary angiogram was undertaken before study product administration to confirm the suitability of coronary anatomy. Ad5FGF-4 (10^{10} viral particles) or matching placebo diluted with normal saline to 5 or 10 ml was administered by intracoronary injections (60% in the left and 40% in the right coronary systems) over a period of 90 s into each target coronary artery or patent bypass graft. In

patients with previous coronary artery bypass surgery, the distribution of the study agent was determined on the basis of the operator's estimation of the distribution of myocardial perfusion. Study drug was administered through subselective coronary artery catheters, placed at least 1 cm distal to the ostium of the target coronary artery. Using contrast injections, angiographers were required to demonstrate that no reflux was present from the injection catheter into the aortic root before and after injection. All films were later reviewed in a blinded manner by one investigator to determine whether or not reflux was successfully prevented.

Patients were hospitalized overnight after the procedure and discharged home the following day. Cardiovascular medications were stabilized for at least six weeks before entry into the study, and patients were urged not to alter the dose of their medication until follow-up was complete.

Follow-up procedures. Outpatient follow-up was undertaken at two, four, and eight weeks post-treatment. History, physical examination, ophthalmic examination (8 weeks), clinical laboratory tests at baseline including hematology, blood chemistry, urinalysis, 12-lead electrocardiogram, and serum FGF-4 levels were performed. Diaries containing information regarding angina attacks and nitroglycerin consumption were collected. Angina class status was recorded and the Duke Activity Status Index questionnaire was administered at four and eight weeks. Adverse events were collected in an ongoing manner.

The main component of the study ended after all patients had completed the perfusion scan at eight weeks. Patients were also seen at 3, 6, and 12 months to record significant adverse events, angina status, and whether they had undergone revascularization.

Scintigraphic technique. The primary method to measure the effect of Ad5FGF-4 on myocardial perfusion was quantitative Tc-99m sestambi myocardial single-photon emission computed tomography (SPECT) performed at rest and then after a standard 6-min intravenous infusion of adenosine at 140 μg/kg/min. Adenosine SPECT was performed at baseline and then repeated at four and eight weeks post-treatment. Anti-ischemic medications and their doses were stabilized before baseline SPECT and were held the morning of each of the three SPECT studies.

The SPECT was performed using a standard rest-stress protocol whereby 8 to 12 mCi of ^{99m}Tc-sestamibi were injected at rest and images were obtained 1 to 2 h after the radiopharmaceutical injection to allow for clearance of ^{99m}Tc-sestamibi from the liver. After the completion of rest imaging, the patient underwent adenosine stress with injection of 25 to 30 mCi of ^{99m}Tc-sestamibi at minute 3 into the infusion. Imaging commenced approximately 1 to 2 h after adenosine stress. A standard gated SPECT acquisition protocol was performed using either single- or double-headed detector systems. For both stress and rest acquisitions, 64 images were obtained over an anterior 180° arc at 25 s/image. For patients weighing more than 250 lbs, a 2-day rest-stress protocol was used with injection of 25 to 30 mCi of ^{99m}Tc-sestamibi on each day.

After image acquisition, the raw data SPECT studies were sent to the core laboratory at Baylor College of Medicine for processing. All images were reconstructed using standard back projection with a Butterworth filter at a 40% cutoff (order 5). The images were then reoriented in the standard three axes for visual review and quantification (4). Gated SPECT acquisition and processing were performed using the standard Cedars protocol and software (8).

The SPECT quantification was performed by one experienced investigator (J.J.M.) who was blinded to treatment assignment. The rest and stress polar maps were independently computer-generated and normalized through use of a circumferential profile analysis. The presence and extent of count reversibility (ischemia) was determined by subtracting the raw data stress polar map from the rest polar map on a pixel-by-pixel basis to generate a "reversibility" polar map. The stress and reversibility polar maps for each patient were statistically compared with a corresponding normal database to determine the stress-induced left ventricular perfusion defect size, defect severity, and the extent of scintigraphic scar and ischemia for each of the three studies per patient. The left ventricular ejection fraction and cardiac volumes were calculated using the quantitative method described by Germano et al. (12).

Statistical analysis. Because no previous experience of the effect of Ad5FGF-4 on perfusion defect size existed, estimation of the sample size was made based on a review of published data comparing nitroglycerin and placebo (7): the primary end point was the change from baseline to week 8 in the stress-related RPDS. A 7% difference in the mean change in RPDS at 8 weeks between Ad5FGF-4 and placebo was assumed. Using an $\alpha = 0.05$ (two-sided) and a standard deviation of 7%, it was determined that a sample size of 45 patients (15 on placebo and 30 on active) would provide ~90% power to detect the difference; for a standard deviation of 8%, power would be ~80% for the same number of patients.

Statistical analysis for the primary (RPDS) and secondary (total perfusion defect size) end points was undertaken according to the intention-to-treat (ITT) principle with the last observation carried forward (LOCF) for missing values. For between-treatment group comparison, continuous variables were analyzed using one-way analysis of variance (ANOVA) or Wilcoxon rank-sum test if data were not normally distributed, and categorical variables were analyzed using chi-square or Fisher exact probability test. A one-way ANOVA model was employed for the between-treatment group comparison of change from baseline in RPDS. The Wilcoxon rank-sum test was applied to the between-treatment group comparison of change from baseline in total perfusion defect size.

The primary end point analysis was also undertaken, excluding data from an outlier in the placebo group who had a 50% (relative) decrease in RPDS (from 31% to 15%) at 4

Table 1. Demographics

	Placebo (n = 17)	Ad5FGF-4 (n = 35)	p Value*
Mean age (yrs)	57 ± 8.6	59 ± 8.1	0.54
Gender, male %	88	89	1.00
Race, Caucasian %	94	94	1.00
Hypertension (%)	71	83	0.47
Diabetes (%)	29	26	1.00
CHF (%)†	53	46	0.77
Previous MI (%)	71	69	0.75
Previous CABG (%)	65	77	0.51
Previous angioplasty (%)	65	46	0.25
Three-vessel disease (%)	41	57	0.38
Angina class			
2 (%)	59	54	1.00
3 (%)	41	46	
Average weekly angina attacks	8 ± 9.1	5 ± 6	0.27
Weekly NTG use	3 ± 3	3 ± 4	0.96
Duration of stable angina (months)	27 ± 32.4	19 ± 24.7	0.35
Mean LVEF (%)	48 ± 10.4	48 ± 10.9	0.98
Mean RPDS (%)	20 ± 8.3	20 ± 9.4	0.91

*p values are based on one-way analysis of variance or Fisher exact test. †CHF was defined based on dyspnea and fatigue (class 1 to 3).

Ad5FGF-4 = adenoviral delivered gene for fibroblast growth factor; CABG = coronary artery bypass graft; CHF = congestive heart failure; LVEF = left ventricular ejection fraction; MI = myocardial infarction; NTG = nitroglycerin; RPDS = reversible perfusion defect size (single photon emission computed tomography sestamibi).

weeks. This analysis was undertaken on clinical grounds. It was thought that a response of this magnitude could not occur unless there was non-compliance for antianginal medication during the pre-treatment period.

The protocol was amended to include an interim analysis when 22 patients had been treated and followed for 8 weeks. The purpose of the interim analysis was to terminate the study based on futility and to permit early planning of a large study. Investigators, patients, core laboratory physician and staff, and sponsor's staff (medical monitor, clinical research scientists, study biostatistician) remained blinded to the results of the interim analysis. Congestive heart failure was defined as fatigue and dyspnea, thrombocytopenia was defined as platelet count below the lower limit of normal, and other laboratory values were considered abnormal if they were not within the normal range.

RESULTS

Patient screening and demographics. Thirteen U.S. hospitals participated. A total of 148 patients were consented, and 53 patients were randomized. Ninety-five patients were excluded, 67 because the RPDS was ≤9%, and 28 for other reasons (cancer, HIV, withdrawal of consent, angina class 1, or other entry criteria not met). One randomized patient underwent angioplasty and dropped out of the study before receiving the study product. Fifty-two patients were randomized and received Ad5FGF-4, 10^{10} viral particles (n = 35) or placebo (n = 17). All patients were followed for at least eight weeks after treatment, but one patient refused to undergo the eight-week post-treatment radionuclide study.

The two groups were well matched (Table 1). The placebo group had a mean duration of angina of 27 months versus 19 months for the Ad5FGF-4 group (p = 0.35). Forty-four percent of patients had class 3 angina and 48% had class 1 to 3 heart failure. The mean left ventricular ejection fraction was 48%. Forty-six percent of the study population had three-vessel coronary artery disease, 44% had two-vessel disease, and 8% had one-vessel disease. Seventy-three percent of the patients had previous coronary artery bypass graft (CABG) surgery, and 52% had previous angioplasty. Twenty-seven percent of the population had type 2 diabetes, 69% had previous myocardial infarction, 79% had hypertension, and 94% had hyperlipidemia.

Baseline scintigraphic and symptomatic profile of patients. The mean baseline total perfusion defect size was 32.4%, with 20% reversible ischemia and 12.5% scar. The mean baseline perfusion stress severity score was 1.2. The two groups were almost identical regarding the baseline perfusion characteristics. The mean weekly number of angina attacks was ~6/week, and the mean weekly nitroglycerin consumption was ~3/week. The most frequent concomitant medications were beta-blockers, calcium channel blockers, long-acting nitrates, angiotensin-converting enzyme inhibitors, and statins. Overall, 82% of patients were using ≥2 antianginal drugs, and these medications remained constant throughout the 8-week post-treatment period. Statin therapy was started within 6 months before study drug administration in 3 (19%) placebo patients and 3 (10%) Ad5FGF-4 patients.

Effect of therapy on myocardial perfusion (Table 2). At 8 weeks post-treatment, Ad5FGF-4 reduced RPDS by 4.2% (21% of the baseline value, p < 0.001), whereas in the placebo group, the reduction of RPDS was only 1.6% (8% of the baseline value, p = 0.32) (Figs. 1 and 2). The difference

Table 2. Perfusion Results Compared With Baseline at 4 and 8 Weeks

	4 Weeks			8 Weeks (With LOCF)		
Compared With Baseline	Placebo (n = 17)	Ad5FGF-4 (n = 35)	p*	Placebo (n = 17)	Ad5FGF-4 (n = 35)	p*
Change in RPDS (ITT)	0.1 ± 6.2	−2.7 ± 4.8	0.08	−1.6 ± 6.4	−4.2 ± 5.6	0.14
Change in RPDS (without 1 outlier on placebo)	1.1 ± 4.8	−2.7 ± 4.8	0.01	−0.8 ± 5.6	−4.2 ± 5.6	<0.05
Change in total PDS	−1.2 ± 5.7	−4.3 ± 5.8	0.03	−2.4 ± 6.5	−4.6 ± 6.0	0.38
Change in severity	0.03 ± 0.10	−0.03 ± 0.11	0.24	0.00 ± 0.09	−0.05 ± 0.11	0.12

*p values are based on one-way analysis of variance or the Wilcoxon rank-sum test if the normality assumption was not met.

Ad5FGF-4 = adenoviral delivered gene for fibroblast growth factor; ITT = intention to treat; LOCF = last observation carried forward; PDS = perfusion defect size; RPDS = reversible perfusion defect size.

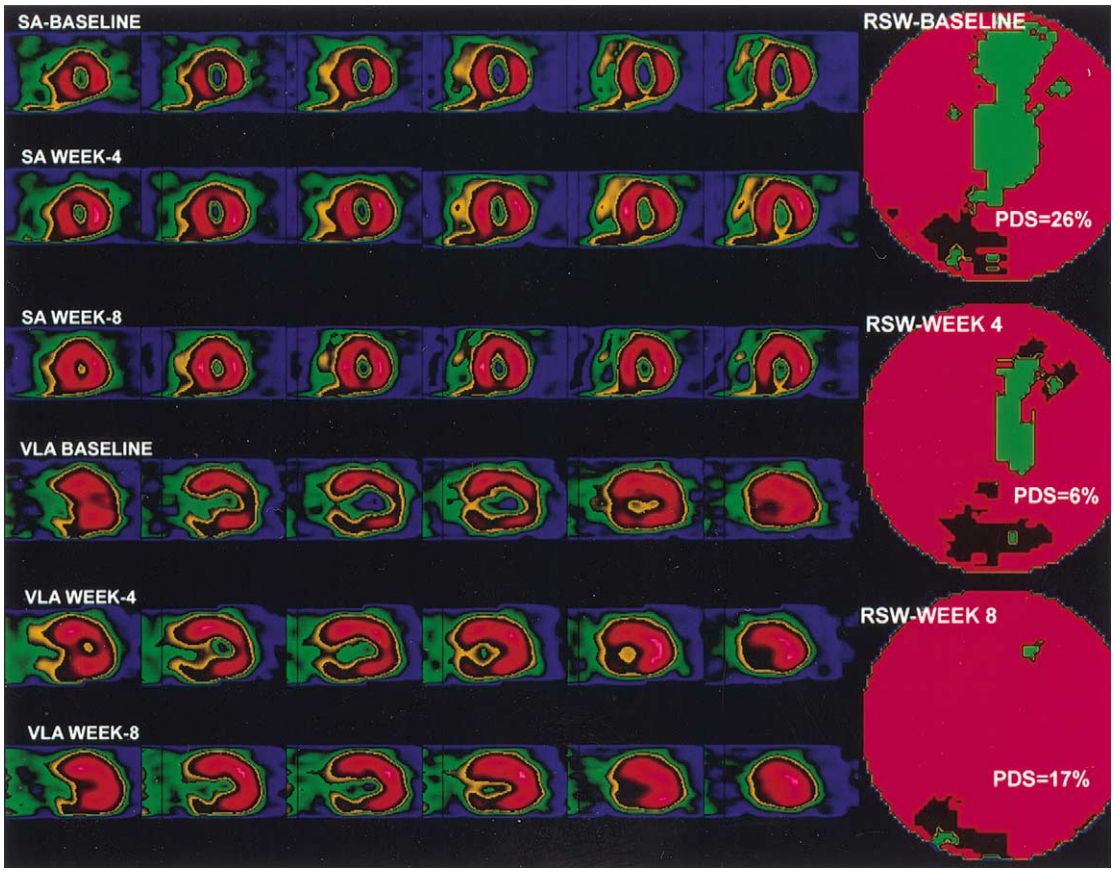

Figure 1. Representative stress only short-axis (SA) **(upper three panels)** and vertical long-axis (VLA) **(lower three panels)** tomographic images of a patient before therapy (baseline) and at week 4 and week 8. The corresponding quantitative polar maps are shown on the **right**. The adenosine-induced ischemic anterior perfusion defect improves dramatically from baseline to week 4 and 8 as reflected by a reduction in perfusion defect size (PDS) from 26% to 17% to 6%. The ischemic **(green)** area of the polar map resolves, whereas the scarred region **(black)** remains unchanged over the eight-week period.

between the reduction in RPDS in the Ad5FGF-4 and placebo groups at eight weeks (primary end point) was not statistically significant ($p = 0.14$). One patient in the placebo-treated group was an outlier, and a 50% reduction compared with the baseline value in RPDS (from 30% to 15%) was observed. This patient was noted to have a much lower resting and peak heart rate during the intravenous adenosine infusion at eight weeks post-treatment (54 beats/min at rest, 71 beats/min at peak) compared with baseline (70 beats/min at rest, 93 beats/min at peak). Therefore, poor compliance with antianginal medication during the pre-treatment period may have accounted for the discrepancy in RPDS. Excluding this patient, a significant difference in RPDS between the two groups was observed at four weeks ($p = 0.01$) and eight weeks after treatment ($p < 0.05$).

Ad-hoc analysis demonstrated that a larger proportion of the Ad5FGF-4 group (51% vs. 35% patients, $p = 0.28$) tended to have a clinically relevant response to treatment (≥5% improvement in RPDS), and fewer patients (6% vs. 35%, $p = 0.01$) had clinically relevant worsening (≥4% increase in perfusion defect) (Fig. 3). Also, in patients with a baseline RPDS <20%, there was a significantly greater improvement, after Ad5FGF-4 ($n = 19$) compared with patients receiving placebo ($n = 10$), 3.8 ± 5.8 versus 1.6 ± 4.4, respectively, $p = 0.009$ (Wilcoxon test). Ejection fraction remained unchanged throughout the study period.

Baseline neutralizing adenoviral antibody titer ≥100 was present in 12 of 35 (33%) Ad5FGF-4 patients. However, perfusion improvements in Ad5FGF-4–treated patients were not affected by the presence or absence of baseline neutralizing antibodies ≥100 (58% and 48% patients improved RPDS ≥5%, respectively, p = NS).

Effect on angina. Improvement in the mean effect of subjective angina parameters (angina class, Duke activity score, weekly angina frequency, and nitroglycerin consumption) was seen in both the placebo and Ad5FGF-4 groups. Freedom from angina was observed in 30% of the Ad5FGF-4 group versus 13% of the placebo group. Forty-three percent of the Ad5FGF-4 group were not taking nitroglycerin eight weeks post-treatment compared with 17% of the placebo group (Fig. 4).

Safety. A total of 145 intracoronary injections of the study product were made, and reflux into the aorta was seen during contrast injection in 10 injections involving 8 (5 active, 3 placebo) patients. However, to opacify the coronary, the contrast injections were made at a higher speed compared with the study drug. Serum FGF-4 levels were

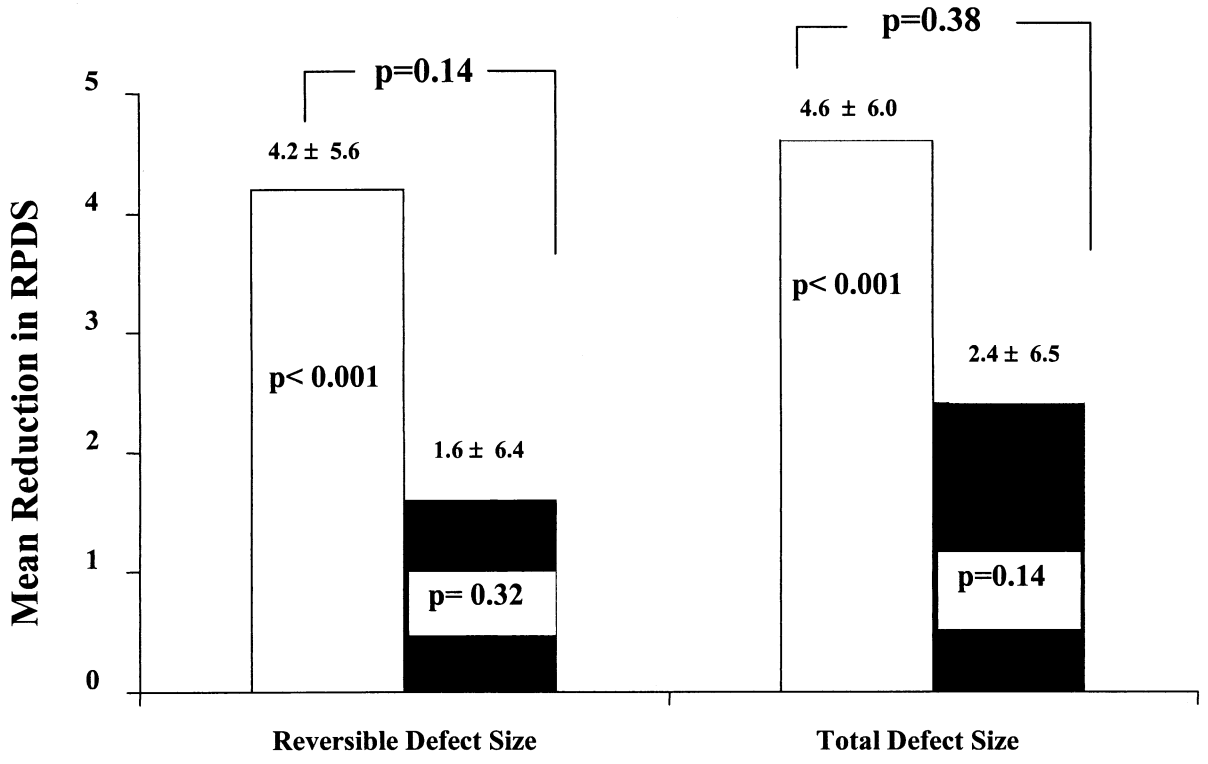

Figure 2. Absolute improvement in perfusion defect size at eight weeks post-treatment: Ad5FGF-4 reduced the total and reversible perfusion defect size (RPDS) by 4.6% and 4.2%, respectively ($p < 0.001$). In the placebo group, the reductions were only 2.4% ($p = 0.14$) and 1.6% ($p = 0.32$), respectively. However, the difference between Ad5FGF-4 and placebo was not significantly different. **White bars** = Ad5FGF-4; **black bars** = placebo.

not detected in any patient at two, four, or eight weeks. The administration of Ad5FGF-4 was well tolerated. There were no deaths, myocardial infarctions, episodes of myocarditis, allergic reactions, or symptomatic hypotension during Ad5FGF-4 administration or at follow-up. Serious adverse events (mostly unrelated) were reported in 13 patients (Table 3). One patient who received Ad5FGF-4 developed transient fever associated with back pain, myalgia, a transient rise in serum total bilirubin to 1.8 mg/dl (direct 0.6 mg/dl), and a transient fall in platelet count to 144,000/μl

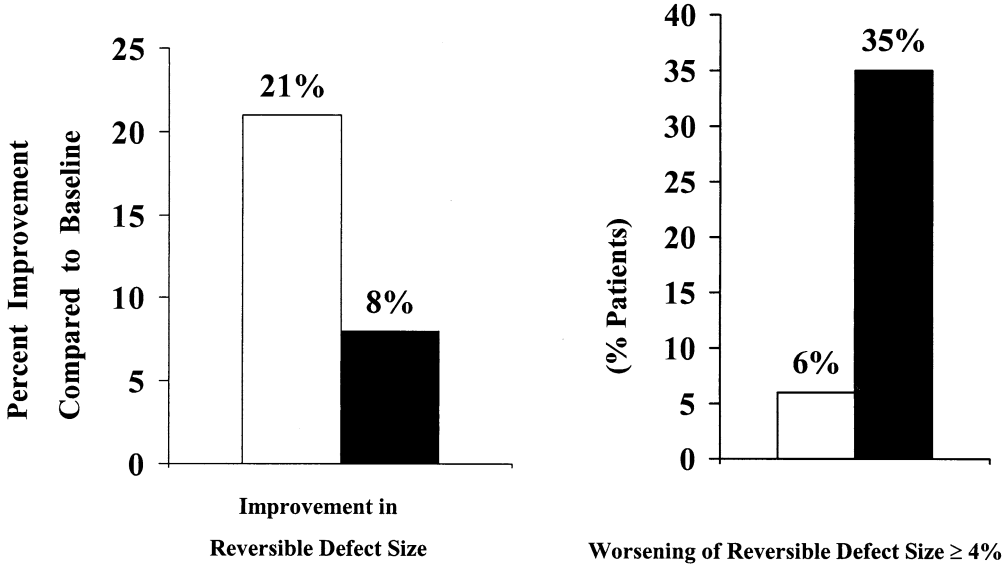

Figure 3. Change in myocardial perfusion at eight weeks: a greater percentage improvement (compared with baseline) in both reversible and total perfusion defect size was seen in the Ad5FGF-4 group compared with the placebo group. Moreover, a greater number of Ad5FGF-4 patients had a clinically relevant improvement in perfusion (>5% improvement), and significantly fewer Ad5FGF-4 patients had clinically relevant worsening (≥4% increase in defect size) of perfusion (6% vs. 35%, $p = 0.01$). **White bars** = Ad5FGF-4; **black bars** = placebo.

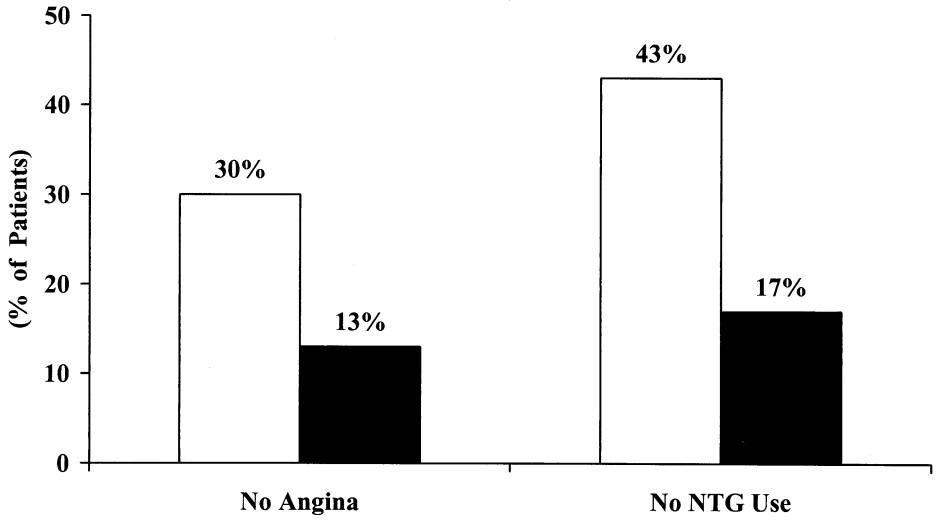

Figure 4. Symptomatic improvements at eight weeks post-treatment: a greater percentage of Ad5FGF-4 patients (compared with placebo) had symptomatic improvement, with 30% of patients having no angina and 43% of patients using no nitroglycerin (NTG) at eight weeks; however, these differences were not significant. **White bars** = Ad5FGF-4; **black bars** = placebo.

(normal range 150,000/μl to 400,000/μl). Both laboratory parameters corrected spontaneously within two weeks. One patient who received Ad5FGF-4 developed a rise in liver enzymes at week 2. The patient tested positive for hepatitis C; however, the study product and its precursor cell and viral banks tested negative for hepatitis C, thus excluding the study product as a possible cause.

Transient, mild (between one and two times the upper limit of normal) increases in serum transaminases (serum glutamic-oxaloacetic transaminase [SGOT] and serum glutamic-pyruvic transaminase [SGPT]) were noted in a few patients. The SGOT levels increased in 3 patients on placebo and in 10 patients on Ad5FGF-4. The SGPT levels increased in 5 patients on placebo and in 10 patients on Ad5FGF-4. Mild CK increases occurred in 5 patients on placebo and in 8 patients on Ad5FGF-4. These were considered to be statin-related. There were no corresponding increases in troponin T and no clinical or electrocardiographic evidence of myocarditis. Platelet counts decreased transiently in six patients (one on placebo, five on Ad5FGF-4). In only one of these the count fell below 100,000/μl (the patient received Ad5FGF-4). This patient had normal marrow histology, and his count returned to normal.

One patient, in the Ad5FGF-4 group, developed transient worsening of congestive heart failure 45 days after treatment. One diabetic patient who received Ad5FGF-4 developed worsening angina and on repeat angiography 154 days after treatment was found to have progression of proximal left anterior descending coronary artery stenosis and underwent angioplasty. A second patient in the active group developed worsening angina and received CABG surgery. Four patients receiving placebo developed worsen-

Table 3. Adverse Events

Serious Events (Related or Unrelated) and Laboratory Abnormalities*	Placebo (n = 17)		Ad5FGF-4 (n = 35)	
	N	%	N	%
Fever with low platelet count	0	0	1	2.8
Worsening or unstable angina†	4	24	2	6
Required angioplasty or CABG	3	18	2	6
Carotid endarterectomy	0	0	1	3
Hepatitis C	0	0	1	3
Worsening CHF	0	0	1	3
Ulcerative colitis	1	6	0	0
Post-laminectomy syndrome	0	0	1	3
Depression	0	0	1	3
Asthmatic bronchitis	0	0	1	3
Increase in SGOT‡	3	18	10	29
Increase in SGPT‡	5	29	10	29
Increase in CK§	5	29	8	23
Reduction of platelet count§	1	6	5	14

*Up to 12 months follow-up for all data available. No significant differences. †p = 0.06 (chi-square). ‡≤2 times the upper limit of normal. §Outside of normal range.

CK = creatine kinase; SGOT = serum glutamic-oxaloacetic transaminase; SGPT = serum glutamic-pyruvic transaminase. Other abbreviations as in Table 1.

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ing angina; three of these four required angioplasty. Overall, the Ad5FGF-4 group had a lower incidence of worsening or unstable angina (6% vs. 24%) or revascularization procedures (6% vs. 18%) compared with the placebo group.

A patient in the placebo group experienced abdominal pain and bloody diarrhea due to ulcerative colitis. One patient in the Ad5FGF-4 group had a skin lesion (squamous cell carcinoma) removed from his nose. He had several skin lesions removed previously; these had been considered premalignant (actinic keratoses), but histopathology was never performed. There was no evidence of retinal neovascularization or angioma formation.

DISCUSSION

Although SPECT myocardial perfusion has been used in other studies of angiogenesis, these trials were either not randomized or blinded (13,14) or showed no improvement in perfusion (1). In addition, these trials utilized a short-lived infusion of protein instead of the gene encoding for the protein. The current study is the first randomized placebo-controlled trial to test the effect of gene therapy for angiogenesis on myocardial perfusion in humans. We found that in a carefully selected patient group with severe coronary artery disease, persistent angina despite optimum antianginal medication, and pharmacologically induced myocardial ischemia, a dose of 10^{10} particles of Ad5FGF-4 injected into the coronary circulation resulted in a clinically relevant (21% increase from baseline value) and significant ($p < 0.001$) improvement in stress-induced myocardial perfusion at eight weeks compared with baseline. However, the increase in stress-induced myocardial perfusion defect, compared with placebo, was not significant (21% relative reduction in defect size for Ad5FGF-4 vs. 8% for placebo, $p = 0.14$). When a single outlier in the placebo group who had a 50% improvement in stress-related perfusion (compared with baseline value) was excluded, a significant benefit was observed ($p < 0.05$). Improvements >25% of baseline values are not expected in the absence of revascularization or antianginal therapy (7,8). The likely cause of this unexpected improvement appears to be irregular compliance to antianginal medications during the baseline period. Moreover, ad-hoc analysis suggested a significant benefit in avoiding the worsening of perfusion ($p = 0.01$) (Fig. 2), as well as a significantly greater decrease in RPDS in patients with baseline RPDS < 20% ($p = 0.009$).

The current study was designed to minimize the biologic variability of ischemia through two approaches. First, patients were required to be on a stable pharmacologic regimen for a baseline period of up to six weeks before administration of the study drug. Second, myocardial perfusion was assessed at a core laboratory in a blinded fashion using a highly reproducible technique. Finally, only patients with clinically relevant reversible perfusion defects of >9% were included (9).

In the current study, intracoronary administration of Ad5FGF-4 was safe and well tolerated. Circulating FGF-4 was not detected. Post-treatment surveillance was intense because of concerns about the possible risks from an exogenously administered gene encoding an angiogenic protein. During the follow-up period, there was no evidence of retinal neovascularization or angioma formation. One skin malignancy was diagnosed in a patient who had previously had premalignant lesions (actinic keratosis), which were excised but not subjected to histopathology. One patient developed worsening angina associated with progression of stenosis that was reported as a possible consequence of placement of the subselective coronary catheter or intracoronary injection, but natural progression in this diabetic patient with three-vessel coronary disease cannot be ruled out.

The current study should be regarded as exploratory. The sample size was small, and effects of angiogenic growth factor therapy on quantitatively determined SPECT defect size have not previously been studied. The statistical assumption used to determine the power of the trial included a 7% absolute difference in perfusion defect size. This assumption was derived from a previous study of transdermal nitrate therapy in patients with stable angina who had no previous myocardial infarction and no regional or global left ventricular function abnormalities at rest. In addition, antianginal drugs were stopped in the majority (80%) of patients in that study (7). Whether this difference actually represents statistical underpowering of the current study based on an effect observed with a treatment with a different mechanism of action (dilation of epicardial conduit vessels) or is a consequence of the spatial resolution of tomographic scintigraphy is not known. Although myocardial scintigraphy using adenosine stress and tomographic sestamibi imaging has been shown to reflect the distribution of blood flow seen with radioactive microspheres, it may not be adequate to detect the growth of fine collateral networks and could have underestimated the effects of an angiogenic growth factor.

Unlike our earlier study (3), the presence of adenoviral neutralizing antibodies at baseline appeared to have no influence on improvement in myocardial perfusion. Finally, recent blinded studies of new therapies for advanced ischemic heart disease have established the magnitude of placebo effect. In each randomized, blinded study of growth factors or transmyocardial laser revascularization, significant improvements in exercise time and anginal status were reported in placebo-treated patients (1–3). Similarly, in the current study, the placebo-treated group had symptomatic improvement, less nitrate use, but non-significant improvement in RPDS.

Given these constraints, the findings of a significant reduction in defect size after treatment with Ad5FGF-4, the trend toward a greater reduction than with placebo, and significantly fewer patients in whom worsening of the ischemic defect size was observed should be regarded as positive signals regarding efficacy of the drug. Consequently,

larger trials exploring the effect of Ad5FGF-4 on clinical outcomes are warranted.

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