

Angiogenic Gene Therapy (AGENT) Trial in Patients With Stable Angina Pectoris

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Background—The angiogenic response to myocardial ischemia can be augmented in animal models by gene transfer with the use of a replication defective adenovirus (Ad) containing a human fibroblast growth factor (FGF) gene.

Methods and Results—The objectives of the Angiogenic GENE Therapy (AGENT) trial were to evaluate the safety and anti-ischemic effects of 5 ascending doses of Ad5-FGF4 in patients with angina and to select potentially safe and effective doses for subsequent study. Seventy-nine patients with chronic stable angina Canadian Cardiovascular Society class 2 or 3 underwent double-blind randomization (1:3) to placebo (n=19) or Ad5-FGF4 (n=60). Safety evaluations were performed at each visit and exercise treadmill testing (ETT) at baseline and at 4 and 12 weeks. Single intracoronary administration of Ad5-FGF4 seemed to be safe and well tolerated with no immediate adverse events. Fever of <1-day duration occurred in 3 patients in the highest-dose group. Transient, asymptomatic elevations in liver enzymes occurred in 2 patients in lower-dose groups. Serious adverse events during follow-up (mean, 311 days) were not different between placebo and Ad5-FGF4. Overall, patients who received Ad5-FGF4 tended to have greater improvements in exercise time at 4 weeks (1.3 versus 0.7 minutes, $P=NS$, n=79). A protocol-specified, subgroup analysis showed the greatest improvement in patients with baseline ETT ≤ 10 minutes (1.6 versus 0.6 minutes, $P=0.01$, n=50).

Conclusions—Results show evidence of favorable anti-ischemic effects with Ad5-FGF4 compared with placebo, and it appears to be safe. Angiogenic gene transfer with Ad5-FGF4 shows promise as a new therapeutic approach to the treatment of angina pectoris. (*Circulation*. 2002;105:1291-1297.)

Key Words: angina ■ angiogenesis ■ gene therapy ■ collateral circulation

Angina pectoris caused by coronary artery disease is a major cause of disability worldwide, affecting nearly 7 million people in the United States alone. Two general approaches to the treatment of angina have proven effective in reducing symptoms and increasing exercise treadmill time: drugs and revascularization by PTCA or CABG. In patients with coronary artery disease, the body's natural angiogenesis in response to repeated bouts of myocardial ischemia can provide collateral blood flow to muscle distal to sites of coronary stenoses. In patients who remain symptomatic, the intrinsic angiogenic response and coronary collateral formation are inadequate to relieve stress-induced ischemia.

Stimulation of angiogenesis presents an attractive additional or alternative approach for the treatment of coronary artery disease. Animal models of coronary artery disease have shown that enhancing coronary collateral formation is

possible.¹⁻⁴ Several clinical trials have been conducted that attempted to relieve angina by increasing coronary collateral formation. Despite early enthusiasm, transmyocardial laser revascularization (TMR) and intravascular angiogenic protein growth factor therapy with basic fibroblast growth factor (bFGF) or vascular endothelial growth factor (VEGF)-165 have been ineffective in placebo-controlled clinical trials. Studies that used intravascular angiogenic proteins may have been unsuccessful because of their short half-life. Conversely, direct injection of FGF protein into the myocardium of patients at the time of CABG resulted in angiographic evidence of enhanced collateral formation; however, effects on exercise time were not evaluated.⁵ Moreover, direct injections into the heart muscle by means of an open thoracotomy are quite invasive.

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TABLE 1. Ad5-FGF4 Product: Total Viral Particles and Total Infectious Units Infused in Each Dose Group

Dose Group	Total Viral Particles	Total Infectious Units
1	3.3×10^8	6.4×10^6 (1.9%)
2	1.0×10^9	2.0×10^7 (2.0%)
3	3.3×10^9	1.3×10^8 (3.9%)
4	1.0×10^{10}	1.9×10^8 (1.9%)
5	3.3×10^{10}	8.8×10^8 (2.7%)

Total infectious units are given as number (percent of viral particles capable of transfection of cells). Infectious units were determined by in vitro bioassay.

Gene therapy may be superior to protein therapy because the vascular endothelium and/or myocardium can incorporate the gene, allowing sustained production of angiogenic protein. Although observational reports of gene transfer experiments in humans have been favorable, they have not been double-blind or randomized.^{6–8} To determine the risk/benefit ratio, larger double-blind, placebo-controlled trials are necessary.

Gene transfer of human FGF 5, delivered by single intracoronary infusion with an adenovirus vector (Ad5-FGF5), was reported to provide sustained (12 weeks) in situ production of growth factors that stimulate angiogenesis, enhance collateral blood flow, and relieve stress-induced ischemia in a chronic coronary occlusion model.² This method of intracoronary delivery resulted in 98% first-pass uptake, providing relative targeting of gene transfer to the heart. To determine the safety and potential clinical efficacy of gene transfer for the treatment of angina, we conducted the Angiogenic GENE Therapy (AGENT) trial, the first multicenter, randomized, double-blind, placebo-controlled trial of a potential angiogenic gene therapy.

Methods

Product

Ad5-FGF4 consists of a human, replication-deficient, serotype 5 adenovirus in which the E1A/E1B genes are replaced by the human FGF4 gene, driven by a cytomegalovirus promoter. Preclinical safety and toxicology testing revealed an absence of toxic effects with Ad5-FGF4, even at a dose of 10^{12} viral particles (vp), which was 100-fold greater than the minimal dose (10^{10} vp) required for a physiological effect in animals. This provided an adequate safety margin for clinical development. The product was manufactured by Berlex Biosciences, Richmond, Calif, and was free of replication-competent adenovirus. The infectivity of Ad5-FGF4 was not altered by passage through selected angiographic catheters or during contact with Omnipaque contrast. Blinded study product or placebo (vehicle: PBS, 2 mmol/L MgCl₂, 2% sucrose) in 2.5 mL volume was shipped by overnight courier to the treatment site after randomization. Table 1 shows the total viral particles and infectious units actually infused in each dose group.

Study Design and Objectives

Enrolled patients were randomly assigned in a double-blind fashion to placebo or active product in a ratio of 1:3 in 6 ascending doses from 3.3×10^8 to 10^{11} vp in half-log increments, which was delivered by 1-time intracoronary infusions. Since this was the first clinical trial of its kind, the first 3 dose groups received doses below those shown to be effective in preclinical studies (per US Food and Drug Administration guidance). The objectives of the AGENT trial were (1) to evaluate the safety and anti-ischemic effects of ascending doses of Ad5-FGF4 gene transfer in patients with stable exertional angina and (2) to select potentially safe and effective dose(s) for subsequent study. All study sites had the protocol and informed

consent approved by the local institutional review board and local biosafety committee, and procedures followed institutional guidelines. The US Food and Drug Administration and the Recombinant DNA Advisory Committee at the National Institutes of Health also approved the protocol.

Patient Population

Patients 30 to 75 years old with chronic stable angina, Canadian Cardiovascular Society angina class 2 or 3, stable for >2 months, who were able to exercise for at least 3 minutes on an exercise treadmill test (ETT) using the modified Balke protocol, were enrolled. Patients were required to show 1 mm of ST-segment change (horizontal or downsloping) and stop exercise for angina severity of 3 on a scale of 1 to 4 during screening ETT, with exercise time that differed by <25% on 2 consecutive baseline tests on separate days. Patients with 1-, 2-, or 3-vessel coronary artery disease were included, provided that at least 1 proximal major vessel had <70% stenosis. Cardiovascular exclusion criteria included left main stenosis >50%, coronary aorto-ostial stenosis that would prevent catheter placement, ejection fraction <40%, patent bypass grafts, CABG surgery within 1 year, angioplasty within 6 months, TMR, unstable angina, New York Heart Association congestive heart failure class 3 or 4, left bundle-branch block, paced rhythm, Mobitz 2 second-degree or greater heart block, documented life-threatening ventricular arrhythmia, atrial fibrillation, and patients who required immediate revascularization at the time of coronary angiography. Other exclusion criteria included childbearing potential (for women), diabetic retinopathy, suspicion of malignancy, history of malignancy within 10 years except basal cell carcinoma, creatinine clearance <45 mL/min by Cockcroft Gault formula, proteinuria >2+, HIV positivity, immunosuppressive therapy, abnormal liver function, or hepatitis B or C. Routine tests, including hematology, urinalysis, troponin T, CK-MB, blood chemistry, and retinal examination by an ophthalmologist, had to be within normal limits. Screening for occult malignancy included PSA, stool for occult blood, Pap smear, pelvic examination, mammography, complete history and physical, and chest radiograph. Interim analysis of blinded data showed very long baseline exercise times in some patients, which would make any improvement difficult to detect. Accordingly, after cohort 3, investigators were requested to exclude patients with baseline total ETT time >10 minutes. By protocol amendment, additional patients with baseline ETT <10 minutes and ejection fraction >30% or patent bypass grafts were randomly assigned to placebo or a dose of 10^{10} vp (dose group 4) to determine whether such patients should be included in future trials.

Study Procedures

Informed consent was obtained from qualified patients. Patients continued antianginal medications at constant doses throughout screening and follow-up to the extent possible. Patients also had a dobutamine stress echocardiogram at pretreatment and at 4 and 12 weeks of follow-up, but the results were not used for end point evaluation. Patients who qualified had coronary angiography in prespecified views after intracoronary administration of nitroglycerin. Placebo (vehicle) or Ad5-FGF4 was infused over a period of 90 seconds through subselective catheters into all major patent coronary arteries that could be engaged (and bypass grafts in some group 4 patients), divided 30% into the left anterior descending, 30% into the circumflex, and 40% into the right coronary distribution, or slightly modified to match the proportion of myocardium served as estimated by the investigator. Catheter position was recorded on cineangiograms before and after each subselective infusion. During intracoronary infusion of the first vessel, blood was concurrently withdrawn from a pulmonary artery catheter by a constant rate pump bracketing the injection and flush to estimate first pass myocardial viral uptake.

Standard biosafety techniques were used for handling and administration of the product. Follow-up monitoring included a 1-hour venous blood sample to assess virus concentration. Venous blood and pulmonary artery samples were assayed for Ad5-FGF4 as determined by end point dilution infectivity assay on permissive cells (detection limit, 250 infectious units [I.U.]/mL; quantification limit, 1000 I.U./mL). Serial

physical examinations and blood tests were performed at 1, 2, 4, 8, and 12 weeks and at 6 and 12 months. Repeat exercise tests were performed 4 and 12 weeks after treatment. Exercise was terminated if the patient completed all stages or if moderately severe angina (grade 3 of 4), abnormal hemodynamic response, or ST-segment depression ≥ 2 mm was noted. Neutralizing titer for adenovirus antibody (maximal dilution of serum that reduced virus infectivity 10-fold, sensitivity of the assay 1:50 titer) and plasma for FGF-4 protein (ELISA; sensitivity, 50 pg/mL) were measured at baseline and at posttreatment visits out to 12 weeks. Semen analyses for viral DNA was performed at 8 weeks.

Patients and investigators remained double-blinded throughout the trial. As specified in the protocol, the sponsor was unblinded after each cohort for safety and efficacy review. A 2-week wait for safety assessment was included after the first, second, and last patient in each cohort before randomization of the next patient. Serious adverse events of concern were reviewed by an independent safety monitoring board.

Sample Size Considered and Statistical Analysis

The protocol specified pairwise comparisons of each active group versus pooled placebo by ANCOVA with baseline score as a covariate. Since there was no dose response, treated patients were also pooled and compared with the placebo group. Assuming an increase in ETT of 0.45 minutes for the placebo group, it was estimated that 67 patients per group (≈ 400 patients total) would need to be enrolled to demonstrate a 30% difference with 80% power. Since safety considerations for a first human trial allowed only a relatively small number of patients in each dose group, the protocol specified that analyses would be done in an exploratory manner. All statistical testing used 2-sided tests and a 5% significance level. Clinical benefit was specified as total exercise duration and time to angina. The protocol allowed additional patients to be enrolled to increase statistical power if the difference between placebo and active group was $\geq 30\%$; therefore, group 4 was expanded. Data were also analyzed excluding patients with baseline ETT >10 minutes (per protocol amendment). For patients who did not have angina on a posttreatment ETT, total ETT time was used. Quantitative data were summarized as mean $\pm$ SD. Qualitative data were summarized by using proportions, with dichotomous cut-points selected (twice the placebo response) to identify patients with substantial improvement in ETT (defined as $>20\%$ at 4 weeks and $>30\%$ at 12 weeks) and were analyzed by χ^2 or Fisher's exact test. Data are presented as change in ETT (absolute and percent) and as the percent of patients with substantial improvement. A post hoc analysis was performed, separating patients with high baseline neutralizing antibody titers to Ad5 ($>1:100$). Hard copies of the treadmill exercise tests and coronary angiograms were analyzed by core laboratories blinded to treatment. First-pass myocardial extraction of Ad5-FGF4 was estimated by the difference between the known amount injected and measured viral titers in pulmonary artery blood by standard pharmacokinetic methods.

Results

Demographics

The sponsor terminated enrollment after cohort 5 because sufficient information to plan the next studies had been obtained. All 79 patients completed all visits to 12 weeks except for 1 patient in dose group 3 who missed the 12-week visit. Distribution of patients by age, sex, and baseline characteristics are shown in Table 2. There were no significant differences between dose groups and placebo except for duration of angina. Antianginal medication use was similar in all dose groups and was remarkably constant (by trial design; Table 2). The majority of patients were judged to be suitable anatomic candidates for angioplasty or bypass surgery by the core angiography laboratory.

TABLE 2. Demographics and Ejection Fraction

Dose Group	Placebo	Active
Patients	19	60
Men/women (% men)	17/2 (87)	52/8 (90)
Mean age, y	61.8 $\pm$ 7.7	59.1 $\pm$ 8.7
No. patients with		
Previous MI	8 (42)	33 (55)
Prior CABG	4 (21)	14 (23)
Diabetes*	7 (37)	19 (32)
Hypertension	10 (53)	33 (55)
CCS class 2	15 (79)	49 (82)
CCS class 3	4 (21)	11 (18)
Duration of angina, mo	27 $\pm$ 23	56 $\pm$ 70
Antianginal meds/pt†	1.8	1.9
Baseline EF, %	58.9 $\pm$ 12.4	59.1 $\pm$ 10.6
Mean No. vessels with $>70\%$ stenosis	1.4 $\pm$ 0.8	1.5 $\pm$ 0.7
Baseline ETT, min	9.4 $\pm$ 3.6	9.0 $\pm$ 3.6

Values are n (%) or mean $\pm$ SD.

Previous MI indicates previous myocardial infarction by history or ECG; prior CABG, prior coronary artery bypass grafting; CCS class, Canadian Cardiovascular Society angina class; EF, ejection fraction determined on baseline echocardiogram; and antianginal meds/pt, mean number of antianginal medications per patient (eg, β -blocker, Ca^{2+} -channel blocker, long-acting nitrate).

*Diabetics were all type 2.

†Only 3 patients had a change in number of medications for angina over 12 weeks of follow-up.

Vector Distribution

No Ad5-FGF4 was detected in any sample in placebo patients. Ad5-FGF4 detection in pulmonary artery blood varied from detectable (250 IFU/mL) in cohorts 1 and 2 up to a maximum of 2.3×10^4 IFU/mL in 1 patient in cohort 5. The frequency of detection increased in the cohorts with the higher doses (Figure 1). The median estimated first-pass extraction across the coronary circulation in dose group 3 through 5 (where measurements were in the range of detection for the assay) was 87% (range, 0% to 100%), consistent with preclinical results.² No virus was detected in urine. FGF4 protein was not detectable at any time in plasma samples. Semen samples obtained at 8 weeks after treatment (sperm generation time in humans) were collected from 12 patients, and all were negative for Ad5-FGF4 DNA by polymerase chain reaction (PCR) analysis.

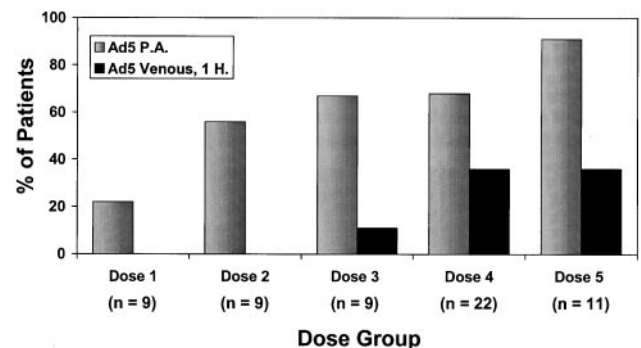


Figure 1. Detection of Ad5-FGF4 in the pulmonary artery during intracoronary infusion and in venous blood 1 hour after infusion.

TABLE 3. Serious Adverse Events, Medications, and Ad5-FGF Detection

Dose Group	Placebo	All Active
Total patients	19	60
No. patients with		
Procedural SAE	0	0
In-hospital fever	0	3 (5)*
Long-term AE and SAE		
Unstable Angina	4 (21)	7 (12)
Total SAE	4 (21)	15 (25)
Transient ↑ SGOT 2×	0	1 (2)
Transient ↑ SGOT 20×	0	1 (2)
Malignancy†	0	2 (3)

Values are number of patients (%).

AE indicates adverse events; unstable angina, worsening angina or unstable angina treated medically, by PCI, or by CABG; total SAE, total serious adverse events (ie, any rehospitalization during long-term follow-up; includes patients with unstable angina, atrial fibrillation, infection, arthritis, hip replacement, back surgery, malignancy, and back pain occurring from 38 to 346 days after treatment); SGOT, serum glutamic oxaloacetic transaminase; transient ↑ SGOT 2×, 2-fold increase in SGOT (1 patient with an isolated increase in SGOT at 1-week follow-up); transient ↑ SGOT 20×, 20-fold increase in SGOT (1 patient in dose group 3; SGOT normalized at week 4 and has remained normal for 1 year).

*All in dose group 5.

†Deemed unlikely to be causally related; both in dose group 4.

Safety

In general, Ad5-FGF4 seemed to be safe and well tolerated.

Procedural and In-Hospital Safety

There were no significant changes in heart rate or blood pressure during product administration. There were no allergic reactions or adverse events during the intracoronary infusion of Ad5-FGF4. All patients had complete administration of the product. Review of the angiograms by the core laboratory for catheter placement revealed that the coronary catheter was displaced during subselective infusion in 4 active and 3 placebo patient arteries. In these 7 patients, some of the infusion may have been delivered into the aortic root instead of the target vessel. Transient mild temperature elevation within 24 hours of product administration developed in 3 patients in dose group 5. Only one of these patients required an extra day of hospitalization for fever.

Long-Term Safety

Mean follow-up was 311 days (range, 57 to 399). There was no evidence of myocarditis either immediately after administration or at follow-up. There were no significant elevations in cardiac enzymes out to 12 weeks of follow-up, no signs or symptoms of new heart failure, and no reduction in cardiac function by echocardiography. There were no significant neovascular changes on retinal examination after treatment in any patient.

Serious adverse events are listed in Table 3. Eleven patients were hospitalized for worsening of angina between 64 and 364 days after product administration (Table 3). Seven of 60 patients (12%) had received active treatment and 4 of 19 patients (21%) had received placebo ($P=NS$). One patient in active dose group 4 had unstable angina at 145 days, and repeat angiography

demonstrated a left main thrombus. While awaiting bypass surgery, the patient had cardiac arrest and died. One patient in active dose group 1 had a transient cerebral ischemic attack at 38 days treated by carotid endarterectomy.

With regard to the possibility of hepatotoxicity, 1 patient in dose group 3 developed asymptomatic elevation in SGPT to 718 U/L (normal ≤ 34 U/L) at 1 week after treatment that returned to normal by 4 weeks and has remained normal after 1 year of follow-up. The patient's bilirubin remained normal. Only one other patient (dose group 4) had transient elevation of liver enzymes >2 times the upper limit of normal (SGPT elevation to 93 U/L at week 1).

Two patients who had received active treatment were diagnosed with malignancy. One 68-year-old man presented with metastatic colon cancer at 267 days and eventually died of metastatic disease. A renal tumor was also discovered. He had 3 first-degree relatives with colon cancer and most likely had hereditary nonpolyposis colon cancer. Although an effect of product administration on clinical course cannot be excluded, the cancer was considered unlikely to have been caused by the product, and PCR assay conducted on the tumor was negative for Ad5-FGF4 DNA. A second patient had symptoms at 69 days after product administration; a brain tumor was diagnosed by biopsy (glioblastoma multiforme). The neurosurgeon and pathologist noted no excessive tumor vascularity. An independent neurological oncologist review indicated that the tumor was almost certainly present at the time of product administration, on the basis of size and doubling time. The tumor was negative for Ad5-FGF4 by PCR and reverse transcription-PCR. The majority of treated patients had a rise in neutralizing antibody titer to adenovirus, and there was no increase in placebo patients. Baseline titers ranged from 0 to 1:3200; titers increased to an equivalent extent in all dose groups up to a maximum of 1:25 600. There was no apparent association between ETT times or adverse events and a rise in antibody titer. Overall, Ad5FGF-4 used in doses up to 3.3×10^{10} vp seemed to be well tolerated, with no major safety concerns.

Effect on ETT Time

The absolute ETT times and percent change for each dose group and placebo are shown in Table 4. No significant dose response in anti-ischemic effect was observed. Overall change in total exercise time for all dose groups pooled and placebo is shown in Figure 2. A post hoc analysis of increases in exercise time when all treated dose groups were pooled at 4 weeks (1.34 minutes) and at 12 weeks (1.67 minutes) was not significantly greater than placebo (0.68 and 0.98 minutes). We tested the effect of censoring the data to include only patients with baseline ETTs of 10 minutes or less (per protocol amendment at dose group 3). In this group, the percent change in ETT for placebo versus treated patients was significantly different at 4 weeks (12% versus 27%, $P=0.01$, [0.6 and 1.64 minutes]) and at 12 weeks (22% versus 30%, $P=0.047$, $n=50$, [1.27 minutes and 1.86 minutes]). The protocol-specified adding of patients was triggered in dose group 4, and a total of 22 were enrolled. There was a significant difference in the proportion of patients with substantial increases in ETT between placebo and dose group 4 at 4 weeks (16% versus 50%, $P=0.046$), and a trend

TABLE 4. Exercise Test Results

	Placebo	Dose Group					All Active
		1	2	3	4	5	
Patients	19	9	9	9	22	11	60
Baseline ETT, min	9.4±3.6	9.5±4.7	10.7±3.4	10.1±4.3	7.3±2.6	8.8±3.0	9.0±3.6
ΔETT at 4 wk	0.7±1.7 (10)	1.9±1.8 (30)	1.4±3.1 (25)	0.5±1.6 (10)	1.5±1.9 (20)	1.2±3.8 (13)	1.3±2.5 (20)
ΔETT at 12 wk	1.0±2.2 (16)	1.7±2.8 (25)	2.2±3.4 (33)	0.6±1.8 (10)	2.0±2.5 (26)	1.4±4.0 (16)	1.7±2.9 (23)
4-Wk 20% incr	3 (16)	5 (56)	4 (44)	2 (22)	11 (50)*	3 (27)	25 (42)
12-Wk 30% incr	4 (21)	4 (44)	2 (22)	2 (25)	10 (45)	3 (27)	21 (36)
12-Wk 30% incr Ab >100	0/2	0/2	0/4	0/0	1/4 (25)	0/4	1/14 (7)
12-Wk 30% incr Ab <100	4/17 (24)	4/7 (57)	2/5 (40)	2/9 (22)	9/18 (50)	3/7 (43)	20/46 (43)†

Values are n (%) or mean±SD (%).

Baseline ETT indicates baseline ETT time in minutes; ΔETT at 4 wk, change in exercise duration at 4-week follow-up and mean % change from baseline; ΔETT at 12 wk, change in exercise duration at 12-week follow-up and mean % change from baseline; 4-Wk 20% incr, number of patients with >20% increase in ETT time (%); 12-Wk 30% incr, number of patients with >30% improvement in ETT time (%); 12-Wk 30% incr ab <100, number of patients with >30% increase in total ETT time at 12 weeks who had baseline neutralizing Ab titer <1:100 (% responders); and 12-Wk 30% incr ab >100, number of patients with >30% increase in ETT time at 12 weeks who had baseline Ab titer >1:100.

* $P<0.05$ for Ad5-FGF4 effect; † $P<0.05$ compared with response rate with Ab titer >100.



continued at week 12 (21% versus 45%). Patients with a low initial neutralizing titer to adenovirus (<1:100) had a significantly better response compared with patients with a high initial titer ($P<0.05$), as shown in Table 4.

Effect on Time to Angina

Trends for improvement in time to angina were not significant. However, when the data excluding patients with baseline ETT time >10 minutes were analyzed by ANCOVA, there was an overall significant effect ($P=0.003$). At 4 weeks, the improvement was 0.7 minutes in placebo and 1.7 minutes in all treated patients. The data must be considered preliminary because the number of patients was small ($n=50$).

Stress Echocardiograms

There were no differences in stress-induced wall motion scores by echocardiography between baseline and 4 or 12 weeks. However, baseline stress-induced regional dysfunction was small (mean=1.2 on a scale of 1=normal to 4=dysfunction), limiting the sensitivity of detecting an interval change.

Discussion

The AGENT trial is the first report of a randomized, double-blind, placebo-controlled trial of an angiogenic gene therapy (Ad5-FGF4) for the treatment of myocardial ischemia. This trial also provides the first human data concerning the safety of intracoronary administration of a replication-defective human adenovirus. Ad5-FGF4 seems to be safe and well tolerated in patients with stable, mild to moderately severe angina when administered by 1-time intracoronary infusion. Although this was a small study involving only 60 treated patients at 5 doses, we successfully identified doses (10^9 and 10^{10} vp) for evaluation in future studies, which are currently underway, on the basis of the apparent safety and anti-ischemic effects observed.

Initial human trials must focus on patient safety. In that regard, it is important to determine whether adverse events were caused by the gene therapy product rather than the patients' underlying disease. This led to an important difference in the design of the AGENT trial compared with other angiogenesis trials. The AGENT trial included a blinded placebo control group and enrolled patients with mild to moderately severe angina. Patients at high risk for adverse events caused by the presence of severe 3-vessel disease, markedly reduced ejection fraction, or severe congestive heart failure were excluded. Furthermore, the majority of patients in this trial had suitable coronary anatomy to undergo a revascularization procedure (PTCA or CABG), whereas most other angiogenesis trials enrolled "no-option" patients.⁶⁻⁸ Additionally, unlike many antianginal trials, in the AGENT trial, long-term antianginal medications were kept constant.

Adenovirus vectors have previously been administered through several other routes of administration in patients (ie, intravenously or by direct tumor injections, inhalation, direct intramyocardial injection, or infusion into the hepatic artery). Previous experience has identified adenovirus administration to be associated with flulike syndromes, including fever and transient elevations of liver function tests.⁹⁻¹¹ At the group 5 dose (3.3×10^{10} vp), 3 patients had 1 day of fever, an effect seen

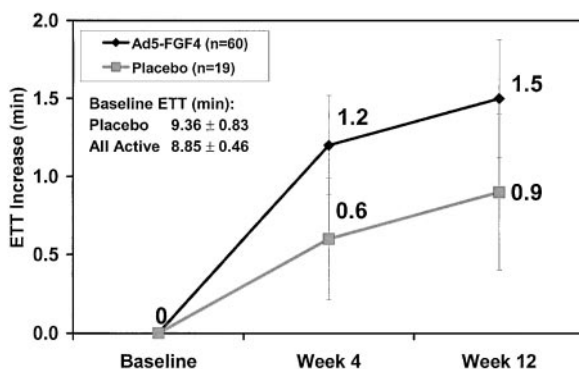


Figure 2. Mean±SEM of change in total ETT time for all active and placebo patients at 4 weeks and 12 weeks after intracoronary infusion of Ad5-FGF4 or placebo.

in other adenovirus trials. Adenovirus has a propensity for hepatotoxicity, but we saw no evidence of dose-related liver toxicity in our patients. An idiosyncratic, transient, potentially product-related elevation in liver enzymes occurred in 1 of 60 active patients and transient mild elevation of 2-fold occurred in a second patient. Thus vigilance for potential liver toxicity should be continued in future trials.

Concerns in delivery of growth factor genes include acceleration of atherosclerosis¹² and angiogenesis at unwanted sites. Importantly, we did not see a difference in unstable angina, revascularization, or worsening in treadmill times in treated patients. Future trials will need to address this further, along with other potential safety issues, in larger numbers of patients. It is reassuring to note the absence of angiomas, retinopathy, and corneal neovascularization. Finally, we saw no evidence of myocarditis or clinically significant changes in hematology parameters.

In animal studies, repeated or sustained infusions of growth factor proteins were required to accelerate collateral growth. The serum half-life of protein growth factors is relatively short, and tissue half-life may be insufficient for the sustained stimulation and multiple cell cycling required for the growth and remodeling of new collateral vessels. Gene transfer with relative cardiac selectivity has the advantage of providing sustained local production of growth factor for several weeks and perhaps longer. The variability in first-pass extraction that we observed may have been due to the volume, speed of infusion, coronary anatomy, the extent of perfusion bed supplied by the vessel, and genetic factors related to viral attachment and internalization. Some growth factors, such as FGF-4, are tightly bound to the proteoglycans of cells where they are produced.¹³ Thus, targeting FGF gene transfer to the heart would be expected to limit systemic toxicity. Furthermore, the absence of circulating FGF-4 in patient plasma with an assay sensitivity (50 pg/mL) that would detect physiologically significant levels (>100 pg/mL FGF-4 for a growth-stimulating effect in cell culture) is also supportive of relatively selective cardiac gene delivery and the safety of the intracoronary infusion of human adenovirus gene therapy products at the doses used.

We observed sufficient evidence of anti-ischemic effects of intracoronary Ad5-FGF4 to select doses of 10^9 and 10^{10} vp for further study, based on a 20 to 30% improvement in ETT. The expected level of improvement in average exercise time after treatment with CABG or angioplasty is 20% to 30%.^{14–16} When the overall response to Ad5-FGF4 is examined qualitatively at these levels, the results suggest that the potential effect of angiogenic gene therapy for angina will be clinically meaningful. Furthermore, we learned of several important factors, such as baseline ETT time and neutralizing antibody titers, that may affect response to angiogenic gene therapy and must be considered in planning future trials. Although the analyses censored for ETT time and antibody titers were not prespecified, there are several plausible reasons for the results. First, patients with exertion limited by angina only after high levels of exercise may remain at the same exercise level despite improvement in myocardial oxygen supply/demand ratio due to other physical limitations and lack of conditioning. Second, results of ETT after treatment must be normalized to the pretreatment value because of the wide distribution of times between patients. In patients

with very long baseline ETT, the percent improvement will be relatively smaller than in more limited patients. Third, the presence of both FGF-4 growth factor production and repeated bouts of ischemia may be required to significantly increase development of collateral vessels. Patients with high exercise capacity may not have ischemia in daily activity frequently enough to augment angiogenesis. Finally, preexisting high neutralizing antibodies to adenovirus serotype 5 from recent infections could reduce the efficacy of gene transfer and expression.^{17,18} One further implication is that efficacy of repeat dosing may be limited by development of neutralizing antibodies until neutralizing antibody titers decrease over time.

There are some limitations to the interpretation of these results. First, we evaluated a selected group of patients with mild to moderately severe stable angina with at least 1 major coronary artery <70% narrowed. Trials of multiple-dose protein growth factor therapy, TMR, and intramyocardial plasmid or adenovirus gene therapy generally have included patients with severe coronary artery disease, usually 3-vessel disease, with no other therapeutic options. Although such patients might improve from increased collateral formation, angiogenic gene therapy would not be expected to grow a new large epicardial conduit artery. Thus, flow-limiting proximal stenosis in all major coronary arteries might continue to limit exercise performance despite enhanced distal collateral formation. Therefore, to test whether angiogenic gene therapy could ameliorate angina pectoris, we excluded patients with severe proximal 3-vessel disease. A second limitation is that inspection of the data did not show an apparent dose-response effect. One likely explanation is that the AGENT trial was not powered to detect a dose response. A second possibility is that a plateau effect was reached at the lower doses. Definitive larger-scale trials based on the information from this phase 1/2 study are needed to rigorously test efficacy and have begun. Subsequent trials will require longer follow-up to confirm a sustained effect and product safety. Finally, we chose exercise-induced angina as measured by ETT duration as the primary measure of efficacy in this exploratory trial and did not seek direct evidence of angiogenesis. Evidence for mechanism of action of Ad5-FGF4 in man would require further clinical trials.

Other uncontrolled, unblinded, gene transfer protocols in early clinical development have used multisite direct myocardial needle injection at thoracotomy of VEGF-165 plasmid DNA, VEGF-2 DNA, or adenovirus containing the VEGF-121 transgene.^{6–8,19} Considering the marked placebo effect that we observed, comparison of these other methods to the intracoronary infusion of adenovirus for angiogenic gene therapy awaits their application in randomized, double-blind, controlled trials.

We conclude that a 1-time intracoronary infusion of Ad5-FGF4 in patients with chronic stable angina appears to be safe and shows evidence supporting efficacy that needs confirmation in a definitive trial. Application of Ad5-FGF4 gene therapy, in addition to the types of patients studied here, might include administration in conjunction with initial diagnostic angiography in patients with symptomatic coronary artery disease or as an adjunct to angioplasty or bypass surgery, especially in patients expected to have incomplete revascularization. Angiogenic gene transfer by a 1-time intracoronary infusion of a replication-deficient adenovirus vector encoding FGF4 shows promise as a

new therapeutic approach for the treatment of patients with angina pectoris.

Appendix

Core Laboratories

ETT: Jonathan Myers, PhD, and Victor Froelicher, MD, Palo Alto VA Health Care System, Palo Alto, Calif; Echocardiography: Thomas Ryan, MD, Duke University, Durham, NC; Angiography: B. Greg Brown, MD, University of Washington, Seattle.

Principle Investigators

The following Principle Investigators enrolled (n) patients in AGENT: Jeffery Brinker, MD, and Jeff Rade, MD (7), Johns Hopkins Hospital, Baltimore, Md; David L. Brown, MD (2), Albert Einstein College of Medicine, Bronx, NY; Cindy L. Grines, MD, and Andrew West, MD (23), William Beaumont Hospital, Royal Oak, Mich; Greg Helmer, MD (9), Minnesota Heart Clinic, Edina, Minn; Stanley Katz, MD (3), North Shore University Hospital, Manhasset, NY; Joon S. Lee, MD (2), University of Pittsburgh, Pittsburgh, Pa; Martin Leon, MD (1), Washington Hospital Center, Washington, DC; Jonathan Marmur, MD (7), Mount Sinai Medical Center, New York, NY; William Penny, MD (8), VA San Diego Healthcare System, San Diego, Calif; Donald Rothbaum, MD (4), Northside Cardiology, Indianapolis, Ind; Matthew W. Watkins, MD (11), Fletcher Allen Health Care, Burlington, Vt; and Robert Wilson, MD (2), University of Minnesota, Minneapolis.

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