Adenovirus-Mediated Gene Transfer of VEGF121 Improves Lower-Extremity Endothelial Function and Flow Reserve

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Background—Vascular endothelial growth factor (VEGF) currently is being evaluated in clinical angiogenesis trials involving patients with peripheral arterial disease. We hypothesized that delivery of VEGF to the skeletal muscle of the lower extremity using an adenoviral vector (Ad GV VEGF121.10) would improve peripheral endothelial function. Accordingly, we investigated lower-extremity endothelial function in patients enrolled in a Phase I adenovirus-mediated gene delivery trial of VEGF121.10.

Methods and Results—Blood flow to the index extremity was measured by thermodilution at baseline and 30 days after administration of Ad GV VEGF121.10, in response to the infusion of endothelium-dependent and -independent agonists (acetylcholine and nitroglycerin, respectively) into the ipsilateral femoral artery. There was no difference in basal flow before or after treatment with AdGV VEGF121.10. In response to acetylcholine (150 μg/min and 300 μg/min), there was a 0.9-fold (0.33 ± 0.03 to 0.32 ± 0.03 L/min) and 1.2-fold (0.33 ± 0.03 to 0.49 ± 0.02 L/min) change in flow before AdGV VEGF121.10 treatment. After AdGV VEGF121.10 treatment, flow increased 2.4-fold (0.31 ± 0.04 to 0.73 ± 0.08 L/min) and 2.3-fold (0.31 ± 0.04 to 0.7 ± 0.08 L/min), respectively (P < 0.05 before AdGV VEGF121.10 treatment versus after AdGV VEGF121.10 for both doses). Infusion of nitroglycerin resulted in a 1.8-fold increase in flow before AdGV VEGF121.10 (0.33 ± 0.03 to 0.58 ± 0.06 L/min) compared with a 2.4-fold increase (0.31 ± 0.04 to 0.73 ± 0.09 L/min) after AdGV VEGF121.10 (P = NS before AdGV VEGF121.10 Versus after AdGV VEGF121.10). Lower-extremity flow reserve increased in all patients in response to at least 1 dose of acetylcholine. Peak walking times increased concomitant with improvement in endothelial function.

Conclusions—Adenoviral gene transfer of VEGF121.10 appears to modulate endothelial function and lower-extremity flow reserve in patients with peripheral arterial disease. (Circulation. 2001;104:753-755.)

Key Words: gene therapy • angiogenesis • growth substances • endothelium • nitric oxide

Vascular endothelial growth factor (VEGF) enhances endothelium-dependent vasorelaxation through a transcriptionally mediated upregulation of the nitric oxide synthase (NOS) gene. Indeed, administration of VEGF protein and gene transfer of the 165–amino acid isoform of VEGF ameliorate endothelial dysfunction. VEGF-induced improvement in endothelial function in preexisting collaterals and conduit vessels may represent an additional mechanism of flow improvement above and beyond any angiogenic effect this growth factor may have.

A number of current gene therapy protocols use replication-deficient adenoviral vectors for the delivery of angiogenic growth factors. The vector is delivered directly to the skeletal muscle or the myocardium in these protocols, with the objective being heightened regional expression. Whether skeletal muscle expression of VEGF results in improvements in vessel wall endothelial function in humans is unknown. Furthermore, it is not known how long these effects may last. To test this hypothesis, we administered an adenoviral vector encoding the 121–amino acid isoform of VEGF (AdGV VEGF121.10) to the skeletal muscle of the lower extremity in patients with peripheral atherosclerotic disease (PAD). We then evaluated its effects on lower-extremity endothelial function and limb flow reserve before and 4 weeks after gene therapy.

Methods

Patient Selection

Patients with disabling intermittent claudication (IC) or rest pain/limb threat (RP) who were part of a Phase I trial to evaluate the safety and efficacy of AdGV VEGF121.10 in patients with PAD were invited to participate in this substudy. The protocol was approved by the University of Michigan Institutional Review Board. All 6

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patients (4 with IC and 2 with RP) enrolled in the Phase I trial consented to participate in the substudy. Data from 5 patients were analyzed (1 patient with RP required an amputation before the 30-day time point). Patients with significant iliac occlusive disease were excluded by a prespecified protocol exclusion. All patients had angiographic evidence of superficial femoral artery and/or infrapopliteal disease in the index limb. Vasoactive medications were withheld for 12 hours before study, and all long-acting vasoactive medications were withheld for ≥24 hours.

Studies were performed 1 day before and ∼30 days after gene therapy. Patients received between $4 \times 10^{10}$ and $4 \times 10^{10}$ particle units of the vector delivered by intramuscular injections to the skeletal muscles of the lower limb. Injections were made in different locations in each patient, dependent on the site of occlusion and the area of desired collateralization.

**Vector**

The vector used in this protocol was an E1a-, partial E1b-, partial E3 vector, with an expression cassette in the E1 region and a cytomegalovirus promoter/enhancer controlling the cDNA.

**Determination of Peripheral Endothelial Function**

All studies were performed in the morning at approximately the same hour with follow-up studies 30 days after treatment. After cannulation of the right femoral artery and vein with 5F sheaths (Cordis Laboratories, Inc.), a custom-designed 5F double-lumen thermodilution catheter (Baxter Scientific, Edwards Division) to measure leg blood flow (LBF) was inserted into the venous sheath, as has been described previously.$^8$ Heart rate and blood pressure were monitored continuously. A 4F introducer was used to deliver drugs through the central lumen of the arterial sheath while blood pressures were monitored through the side port. LBF was determined by injecting 1 mL of normal saline into the thermodilution catheter with flow displayed by a computer in L/min. After a 10-minute resting phase, acetylcholine was infused into the femoral artery at 150 g/min. After a 10-minute resting phase, nitroglycerin was infused for 4 minutes at a dosage of 100 g/min, followed by LBF measurements. All drugs were infused at the same flow rate. LBF measurements were performed every 30 s for a total of 10 determinations 4 minutes into each dose.

**Rest and Exercise Ankle Brachial Index Determinations**

A technician who was unaware of the treatment status of the patients performed rest and exercise ankle brachial index (ABI) determinations. A standardized Gardner protocol was used for determining peak walking time (PWT).

**Statistics**

All values are expressed as mean ± SEM. Flows before and after gene therapy for each intervention were compared by paired t tests. Statistical significance was set at P<0.05.

**Results**

The Table summarizes the vector dose used, ABI determinations, and PWT data at baseline for all patients. The mean age of the group was 65 ± 6 years. The average ABI for the group was 0.43 ± 0.09. Of the 5 patients evaluated, 4 had IC and 1 had RP. This included 2 patients with diabetes and 2 patients with previous revascularization attempts. Three patients developed leg edema around the injection site within a week of administration. One patient developed a generalized body rash on day 2, whereas another developed a localized reaction at the injection site on day 4, both of which resolved spontaneously.

Baseline flow to the limb remained approximately the same before and after Ad_{GV} VE GF121.10 therapy (0.33 ± 0.03 versus 0.31 ± 0.04 L/min). In response to acetylcholine infusion at 150 μg/min, there was a 0.9-fold change in flow (0.33 ± 0.03 to 0.32 ± 0.03 L/min) before Ad_{GV} VE GF121.10 treatment versus a 2.4-fold increase in flow (0.310 ± 0.04 to 0.730 ± 0.10 L/min) after Ad_{GV} VE GF121.10 treatment (P<0.05). Infusion of 300 μg/min of acetylcholine increased flow 1.2-fold (0.33 ± 0.03 to 0.490 ± 0.02 L/min) before Ad_{GV} VE GF121.10 therapy, whereas flow increased 2.3-fold (0.31 ± 0.04 to 0.7 ± 0.08 L/min) after Ad_{GV} VE GF121.10 therapy (P<0.05) (Figure 1A). The endothelium-independent agonist nitroglycerin resulted in a 1.8-fold increase in flow before Ad_{GV} VE GF121.10 (0.33 ± 0.03 to 0.58 ± 0.06 L/min) compared

**Figure 1.** A, Effect of graded acetylcholine infusions (150 and 300 μg/min; A150 and A300) on absolute lower-extremity blood flow before and after treatment with Ad_{GV} VE GF121.10. B, Effect of infusion with the endothelium-independent dilator nitroglycerin (100 μg/min) on blood flow to the lower extremity at baseline and 30 days after administration of Ad_{GV} VE GF121.10.
end of the 30-day period are shown in the Table.

PWTs in all evaluable patients at the dose of acetylcholine. PWTs in all evaluable patients at the

individual patient (flow reserve). As expected in a heterogeneous patient population, there was wide variation in responses to acetylcholine. At the end of 30 days, almost all patients showed some improvement in response to at least 1

lower-extremity endothelial function. These effects were noted after 4 weeks in a relatively older group of patients with advanced PAD, suggesting that VEGF may have an important and durable influences on endothelial function in this patient population. Improvements in endothelial function were paralleled by favorable changes in PWTs in patients who could undergo exercise testing.

As such, this study extends previous observations in animal models, which suggested that recombinant VEGF delivered as protein or gene therapy may have a favorable impact on flow reserve and endothelial function.5,5 The mechanisms accounting for this effect have been postulated to involve a direct angiogenic effect of VEGF, as well as the effect of VEGF on the NOS pathway. The former effect may indeed be inextricably linked to the latter; studies in cultured endothelial cells and in animal models have demonstrated that NO may be a requirement for the angiogenic effect of VEGF.9–11

At this point, the mechanisms responsible for the sustained effect of VEGF 30 days after administration of Ad 

Although there seemed to be a trend toward improvement in response to nitroglycerin, this trend was not significant. The reasons for this are unclear at this point. Potential explanations include a subtle effect that may become apparent with larger sample size, or a lack of true collateral vessel enhancement with VEGF.10

It is interesting that some patients had remarkable degrees of improvement in PWT, with concomitant improvement in endothelial function, whereas others did not experience the same level of improvement despite positive changes in endothelial function. Because of the small number of patients, however, it is not possible to make accurate conclusions regarding the optimal titer of vector or the dose dependency of endothelial function.

The limitations of the study are that these data were obtained in an open-label trial, and we did not provide direct evidence of gene expression. Nonetheless, the data strongly suggest that VEGF may have an important and sustained effect on blood flow to an organ through its effect on endothelial function, in addition to any collateral vessel enhancement that may occur because of the angiogenic properties of VEGF.

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References


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