Adenovirus-Mediated Gene Transfer of VEGF\textsubscript{121} Improves Lower-Extremity Endothelial Function and Flow Reserve

Sanjay Rajagopalan, MD; Manan Shah, BS; Ann Luciano, RN; Ronald Crystal, MD; Elizabeth G. Nabel, MD

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\textsubscript{GV}VEGF\textsubscript{121.10}) 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 VEGF\textsubscript{121.10}.

Methods and Results—Blood flow to the index extremity was measured by thermodilution at baseline and 30 days after administration of Ad\textsubscript{GV}VEGF\textsubscript{121.10} 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 Ad\textsubscript{GV}VEGF\textsubscript{121.10}. In response to acetylcholine (150 $\mu$g/min and 300 $\mu$g/min), there was a 0.9-fold (0.33\( \pm \)0.03 to 0.32\( \pm \)0.03 L/min) and 1.2-fold (0.33\( \pm \)0.03 to 0.49\( \pm \)0.02 L/min) change in flow before Ad\textsubscript{GV}VEGF\textsubscript{121.10} treatment. After Ad\textsubscript{GV}VEGF\textsubscript{121.10} treatment, flow increased 2.4-fold (0.31\( \pm \)0.04 to 0.73\( \pm \)0.10 L/min) and 2.3-fold (0.31\( \pm \)0.04 to 0.78\( \pm \)0.08 L/min), respectively (\( P < 0.05 \) before Ad\textsubscript{GV}VEGF\textsubscript{121.10} treatment versus after Ad\textsubscript{GV}VEGF\textsubscript{121.10} for both doses). Infusion of nitroglycerin resulted in a 1.8-fold increase in flow before Ad\textsubscript{GV}VEGF\textsubscript{121.10} (0.33\( \pm \)0.03 to 0.58\( \pm \)0.06 L/min) compared with a 2.4-fold increase (0.31\( \pm \)0.04 to 0.73\( \pm \)0.09 L/min) after Ad\textsubscript{GV}VEGF\textsubscript{121.10} (\( P = \text{NS} \) before Ad\textsubscript{GV}VEGF\textsubscript{121.10} versus after Ad\textsubscript{GV}VEGF\textsubscript{121.10}). 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 VEGF\textsubscript{121.10} 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\textsuperscript{1} through a transcriptionally mediated upregulation of the nitric oxide synthase (NOS) gene.\textsuperscript{2,3} Indeed, administration of VEGF protein and gene transfer of the 165-amino acid isoform of VEGF ameliorate endothelial dysfunction.\textsuperscript{4-7} 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 (Ad\textsubscript{GV}VEGF\textsubscript{121.10}) 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 Ad\textsubscript{GV}VEGF\textsubscript{121.10} 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
patients (4 with IC and 2 with RP) enrolled in the Phase 1 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. Vasooactive medications were withheld for 12 hours before study, and all long-acting vasooactive 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 $4 \times 10^{9}$ 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 Elα- partial Elb, partial E3 vector, with an expression cassette in the El 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 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 AdGV-VEGF121.10 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 AdGV-VEGF121.10 treatment versus a 2.4-fold increase in flow (0.31±0.04 to 0.73±0.10 L/min) after AdGV-VEGF121.10 treatment (*P*<0.05). Infusion of 300 μg/min of acetylcholine increased flow 1.2-fold (0.33±0.03 to 0.49±0.02 L/min) before AdGV-VEGF121.10 therapy, whereas flow increased 2.3-fold (0.31±0.04 to 0.7±0.08 L/min) after AdGV-VEGF121.10 therapy (*P*<0.05) (Figure 1A). The endothelium-independent agonist 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...
Figure 2. Percent change in LBF (as a percentage of baseline flow) in response to infusion of acetylcholine at 150 and 300 μg/min (A150 and A300) for each individual patient (numbered 1 through 5) before and after treatment with AdGV VEGF121.10.

with a 2.4-fold increase (0.31±0.04 to 0.73±0.09 L/min) after AdGV VEGF121.10 (Figure 1B).

Figure 2 depicts lower-limb blood flow in response to infusion of acetylcholine at 150 and 300 μg/min in the treated limb, expressed as a percent of baseline blood flow for each 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 dose of acetylcholine. PWTs in all evaluable patients at the end of the 30-day period are shown in the Table.

Discussion
The present study demonstrates in humans that adenoviral-mediated gene transfer of VEGF121.10 may favorably influence 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 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 AdGV VEGF121.10 are merely speculative. It is possible that VEGF, through its angiogenic effect as well as through its direct effect on NOS, improves blood flow in the short term. Improved short-term blood flow in turn leads to sustained improvement in endothelial function over the long term, owing to amelioration of shear stress and flow characteristics in the ischemic bed.

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 VEGF121.10.

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.

Acknowledgment
This work was supported in part by National Institutes of Health grant M01-RR00042 to the University of Michigan Clinical Research Center.

References
Adenovirus-Mediated Gene Transfer of VEGF₁₂₁ Improves Lower-Extremity Endothelial Function and Flow Reserve
Sanjay Rajagopalan, Manan Shah, Ann Luciano, Ronald Crystal and Elizabeth G. Nabel

Circulation. 2001;104:753-755
doi: 10.1161/hc3201.095192
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2001 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/104/7/753

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation is online at:
http://circ.ahajournals.org/subscriptions/