Brief Rapid Communications

Effect of Intracoronary Recombinant Human Vascular Endothelial Growth Factor on Myocardial Perfusion

Evidence for a Dose-Dependent Effect

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Background—Animal models of therapeutic angiogenesis have stimulated development of clinical application in patients with limited options for coronary revascularization. The impact of recombinant human vascular endothelial growth factor (rhVEGF) on myocardial perfusion in humans has not been reported.

Methods and Results—Fourteen patients underwent exercise (n=11), dobutamine (n=2), or dipyridamole (n=1) myocardial perfusion single photon emission CT (SPECT) before as well as 30 and 60 days after rhVEGF administration. After uniform processing and display, 2 observers blinded to the timing of the study and dose of rhVEGF reviewed the SPECT images. By a visual, semiquantitative 20-segment scoring method, summed stress scores (SSS) and summed rest scores (SRS) were generated. Although the SSS did not change from baseline to 30 days (21.6 versus 21.5; P=NS), the SRS improved after rhVEGF (13.2 versus 10.4; P<0.05). Stress and rest perfusion improved in >2 segments infrequently in patients treated with low-dose rhVEGF. However, 5 of 6 patients had improvement in >2 segments at rest and stress with the higher rhVEGF doses. Furthermore, although neither the SSS nor the SRS changed in patients treated with the low doses, the SRS decreased in the high-dose rhVEGF patients at 60 days (14.7 versus 10.7; P<0.05). Quantitative analysis was consistent with the visual findings but failed to demonstrate statistical significance.

Conclusions—Although not designed to demonstrate rhVEGF efficacy, these phase 1 data support the concept that rhVEGF improves myocardial perfusion at rest and provide evidence of a dose-dependent effect. (Circulation. 2000;101:118-121.)

Key Words: growth substances ■ tomography ■ angiogenesis

Limited options for myocardial revascularization are available for many patients with severe atherosclerotic heart disease. Therapeutic angiogenesis, or the growth of new nutrient vessels, has been demonstrated in a variety of experimental preparations with vascular endothelial growth factor (VEGF) and basic fibroblast growth factor1–5 and in preliminary clinical trials with angiogenic growth factors.6–8 The preliminary findings of angiographic improvement in collateral blood flow and relief of ischemia and symptoms offer the possibility of new alternatives for treatment.

Recently, a phase 1 trial was completed demonstrating overall safety and tolerability of recombinant human (rh) VEGF in humans. As part of this investigation, all patients underwent stress and rest myocardial perfusion imaging before and after intracoronary administration of rhVEGF, and these serial SPECT perfusion data form the basis of this report.
where uniform processing and display were performed. Image interpretation was by consensus of 2 readers blinded to the type of study (baseline or follow-up), dose of rhVEGF, and clinical data. A semiquantitative 20-segment scoring system was used, with a range of scores from 0 to 4 (0=normal activity, 4=no activity), as previously described. These scores were added to yield a summed stress and a summed rest score. An additional interpretation was performed whereby the studies were evaluated side-by-side. Quantitative analysis was also performed by a polar projection–based method (3D MSPECT), with a threshold for abnormalcy set at $2.5 \text{SD}$ from normal patient distribution; results were calculated as both severity (% of total) and extent (number of SDs), with the product of these measures providing a total defect score.

Continuous data were expressed as mean±SD and compared by ANOVA. Comparisons in discrete variables were made with Fisher’s exact tests. Thirty- and 60-day comparisons with baseline values were performed with the Bonferroni method to adjust for multiple pairwise comparisons. A value of $P<0.05$ was considered statistically significant.

**Results**

Serial SPECT imaging was performed in 10 patients with $^{201}$TI, in 3 patients with $^{99m}$Tc-sestamibi, and in 1 with a dual-isotope protocol. The stress procedure was treadmill exercise in 11 patients, dobutamine infusion in 2 patients, and dipyridamole in 1 patient.

In the direct, blinded comparison of baseline and 30- and 60-day post-rhVEGF images, 10 of 14 patients demonstrated improvement on the resting follow-up images, including 5 of the 6 patients receiving rhVEGF at higher doses. An example of improved perfusion after rhVEGF is shown in Figure 1. One patient manifested no difference, and 3 had worsening of the perfusion pattern after rhVEGF treatment.

By segmental scoring, a mean of 2.5 to 3.4 segments showed improved perfusion after rhVEGF, as shown in the Table. The magnitude of change was greater with the higher doses of VEGF and was most prominent in the resting images.

The summed stress score failed to demonstrate improvement at 30 days ($21.6\pm10.1$ versus $21.5\pm7.8; P=\text{NS}$) or 60 days ($21.4\pm9.8; P=\text{NS}$) after rhVEGF therapy. However, the summed rest score decreased significantly from baseline ($13.2\pm5.8$) to 30 days after VEGF ($10.4\pm7.1; P<0.05$), although this effect did not persist at 60 days ($12.6\pm7.0; P=\text{NS}$). Quantitative analysis revealed similar findings, with trends for reduced total defect score at day 30 for both stress ($112$ versus $102; P=0.14$) and resting ($70$ versus $57; P=0.19$) images.

When the 14 patients were subgrouped on the basis of dose administered, once again no effect was noted in the stress images between baseline and 60 days, either at low dose ($22.5\pm12.6$ versus $23.6\pm10.6; P=\text{NS}$) or high dose.
(20.3±6.5 versus 18.5±8.5; P=NS), a finding confirmed by quantification. However, a dose-related improvement occurred in resting myocardial perfusion (Figure 2); low-dose rhVEGF was associated with no change in perfusion (12.1±6.2 versus 14.1±8.2; P=NS), but higher doses were associated with a significant improvement in perfusion (14.7±5.4 versus 10.7±4.9; P<0.05). Improvement in ≥2 segments was noted in 1 and 2 of the stress and rest studies, respectively, in the 8 patients receiving low-dose VEGF. In contrast, among the 6 patients receiving high-dose VEGF, 5 demonstrated improvement in both stress and resting perfusion in ≥2 segments. Quantitative analysis revealed a decrease in defect score at 60 days for both rest (49 versus 42) and stress (86 versus 66), although these changes were not statistically significant.

Improvement in collateral count density was noted in all 7 patients who underwent serial angiography. The 60-day SPECT studies were improved in 4 of these 7 patients, all of whom received high-dose VEGF; the 3 patients who were treated with low-dose VEGF did not demonstrate scintigraphic improvement.

### Discussion

The present study provides information regarding myocardial perfusion in patients who received rhVEGF in a phase 1 investigation designed to demonstrate safety and tolerability and not powered to test therapeutic efficacy. VEGF improved perfusion in serial SPECT studies, predominantly in the resting images. In addition, a dose-dependent response was noted, because only the higher doses were associated with improved perfusion that persisted at 60 days at rest. The lack of a persistent effect for all patients (60-day summed rest score) may be related to the small sample size or less than ideal perfusion images. Alternatively, progression of disease may be present or a lack of a persistent VEGF effect on coronary flow may be operational.

VEGF has been shown to be uniquely mitogenic for endothelial cells.\(^2,^4\) Because VEGF production and the expression of VEGF receptors is upregulated by hypoxia, angiogenesis is uniquely targeted to areas most deficient in perfusion.\(^1^0\) rhVEGF has been shown to improve blood flow and enhance collateral development in multiple animal preparations.\(^3^–^5\)

Naked plasmid DNA encoding VEGF (phVEGF) has also been used to promote angiogenesis, as initially demonstrated in a rabbit hindlimb ischemia model.\(^1\) Similar results have also been demonstrated in human subjects.\(^6^,^7\) Using direct myocardial injection of phVEGF in 5 patients with chronic myocardial ischemia, Losordo et al\(^7\) demonstrated reduced anginal symptoms, improved collateral flow, and improved perfusion by \(^99\)Tc-sestamibi imaging. However, there was greater improvement in resting perfusion (consistent with the present findings), and the irreversible defects on stress-rest imaging were reduced by 50%, accounting for the majority of change in perfusion.

mRNA expression of both VEGF and its receptors is upregulated by ischemia and hypoxia,\(^1^1\) and the finding that resting rather than stress perfusion is enhanced by rhVEGF suggests that the predominant stimulus for angiogenesis may be present in tissues with persistent and chronic ischemia, as found in patients with reduced resting blood flow. The lack of a substantial effect on stress images after angiogenic therapy is consistent with the observation that collateral vessels, such as those induced by rhVEGF administration, fail to provide the normal degree of maximal coronary flow reserve seen in non–collateral-dependent regions during maximal exercise or with pharmacologically induced vasodilation.\(^1^2\)

In conclusion, this study demonstrates potentially beneficial effects of rhVEGF, as assessed objectively with tomographic perfusion imaging. The predominant effect on resting perfusion appears to be consistent with the need for a chronic hypoxic signal to promote new blood vessel formation. In addition, the present data also provide evidence for a dose-dependent effect. These concepts will require additional validation in larger, prospectively designed clinical trials.

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### References

1. Tsurumi Y, Takeshita S, Chen D, Kearney M, Rossow ST, Passeri J, Horowitz JR, Symes JF, Isner JM. Direct intramuscular gene transfer of naked DNA encoding vascular endothelial growth factor augments col-


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