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EDITORIAL COMMENT

Therapeutic Angiogenesis: Time for the Next Phase*

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Restoring blood flow is the most effective means to limit damage to ischemic tissue. Since the 1960s, surgical placement of bypass grafts for coronary artery disease (CAD) and peripheral vascular disease has effectively and durably restored blood flow to ischemic tissue. Nonsurgical revascularization was introduced clinically starting in the 1970s. Thrombolytic therapy is used to treat acute thrombotic arterial occlusion. Angioplasty combined with stent placement is currently the most frequently applied nonsurgical method of relieving acute coronary ischemia.

Now, therapeutic angiogenesis, the promotion of new vessel growth using vascular growth factors, is the latest and perhaps most exciting strategy for revascularizing ischemic tissue (1–4). Angiogenesis is induced by invasive (surgical or catheter-based approaches) and noninvasive (intramuscular or intra-arterial) delivery of a variety of promoters (5). Vascular endothelial growth factor (VEGF) (6) and basic fibroblastic growth factor (bFGF) (7) are the angiogenic agents in most current clinical studies.

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Four isoforms of VEGF differing in numbers of amino acids are known. The isoforms with 121 and 165 amino acids ($VEGF_{121}$ and $VEGF_{165}$) are being used in human investigations. Vascular endothelial growth factor avidly binds to heparin with a half-life of about 6 min and causes dose-dependent hypotension. Its actions are specific to endothelial cells.

Basic fibroblastic growth factor also binds to heparin. Unlike VEGF, it binds to receptors on fibroblasts and vascular smooth muscle as well as endothelial cells. It has a circulatory half-life of approximately 45 min and also causes a dose-dependent hypotension.

The optimal way to package and deliver these agents is uncertain. They can be given directly as the protein or indirectly as a gene. The advantage of giving VEGF or bFGF as the protein is the ability to achieve precise dosing at known intervals, while the disadvantage is that a large quantity is required because of its short half-life. Delivering the genetic material to host cells that take it up and then produce the growth factor has an innate elegance. Although this approach theoretically enables continuous production of the growth factor, the amount and duration of production is unknown and unregulated. Numerous issues remain concerning the most efficacious and safest method for packaging and delivering the growth factors (5).

ANGIOGENESIS IN ANIMAL MODELS OF ISCHEMIA

Animal studies have established the principle that collateral function improves after administrating angiogenic growth factors using different methods of packaging and delivery.

In dogs given intracoronary injections of bFGF, myocardial angiogenesis had been reported, as evidenced by increased synthesis of endothelial cell DNA, increased collateral vessel density and improved regional blood flow (8). Vascular endothelial growth $factor_{165}$ is also effective. When it was infused continuously over four weeks into the myocardium adjacent to the site of an ameroid constrictor placed on the circumflex artery of swine, blood flow to this region increased at rest and during stress (9). Intravenous injection of these growth factors may not induce angiogenesis as effectively, because of the large volume of dilution and first pass uptake by the lungs (5).

Using the model of hind limb ischemia in the rabbit, intramuscular bFGF (10) and acidic fibroblast growth factor (11), and also intramuscular and intra-arterial $VEGF_{165}$ (12,13), stimulate angiogenesis documented by increased collateral vessel density on angiography, higher perfusion pressure in the distal vessels and increased blood flow. Increased vascular endothelial proliferation also occurs.

CLINICAL STUDIES OF ANGIOGENESIS IN CAD

Observations from four uncontrolled, nonrandomized studies have demonstrated safety and feasibility of VEGF and bFGF with some suggestions of efficacy. Intramyocardial injections of VEGF through a mini-thoracotomy were done in five patients with refractory angina not amenable to surgical or interventional therapy (14). At the time of bypass graft surgery, DNA encoding $VEGF_{121}$ carried by an adenoviral vector was administered into the myocardium of a nonbypassable coronary artery in 21 patients (15), and microcapsules containing FGF were implanted into the epicardial fat of a diseased vessel in eight patients (16). At the time of angiography, 14 patients received either low or high dose VEGF by selective infusion into a coronary artery over 20 min (17). Each of these studies reported reduction in angina and less ischemia or increased collateral vessels assessed by nuclear study, magnetic resonance imaging or coronary angiography. Because of the small number of patients and lack of control groups, the results from these studies need to be considered cautiously.

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Two randomized, controlled series of angiogenic growth factors in individuals with CAD have been reported. Forty patients received an intramyocardial injection of bFGF or placebo into the region of the anastomosis of the internal mammary graft to the left anterior descending artery (18). Angiography performed 12 weeks later was interpreted as showing arterial collaterals in the area of the bFGF injection. No long-term follow-up or objective evidence of improved blood flow was reported. The second study randomized patients to receive one of three doses of $VEGF_{165}$ or placebo intracoronary followed by intravenous infusion on days 3, 6 and 9 (19). The exercise time of the treated and placebo group improved equivalently. The study was terminated due to the absence of a positive effect of the VEGF (5). Although the lack of benefit may reflect the route of administration chosen (IV), the improvement in the nontreated patients noted in this study reinforces the crucial need for a placebo-controlled group in clinical studies.

CLINICAL STUDIES OF ANGIOGENESIS IN PERIPHERAL VASCULAR DISEASE

Isner and colleagues (20) first injected naked human plasmid DNA encoding $VEGF_{165}$ into 10 affected limbs of nine patients. Vascular endothelial growth factor expression peaked from one to three weeks after the gene transfer. After four weeks, the ankle-brachial index increased by 30% (0.33 ± 0.05 to 0.43 ± 0.04). This improvement was sustained at 12 weeks. Blood flow measured by magnetic resonance imaging improved in 8 of 10 limbs with angiographic evidence of new collateral formation observed in 7 of 10 extremities.

The article by Lazarous et al. (21) in this issue of the *Journal* is the first report of bFGF administered intra-arterially in humans. In this phase I study, subjects with intermittent claudication were randomly assigned to placebo group ($n = 6$) or bFGF at 10 μg/kg once ($n = 4$), 30 μg/kg once ($n = 5$) or 30 μg/kg given on two consecutive days ($n = 4$). The agent was infused into the artery of the most severely affected leg. The individuals were monitored closely over time for known and potential side effects of bFGF. The rate of infusion was adjusted to avoid hypotension that occurred in the first subject that received bFGF. A second subject had a fall in platelet count from a low normal value ($150{,}000/mm^3$ to $124{,}000/mm^3$), while a third person with no history of arrhythmia developed atrial fibrillation with spontaneous conversion 12 h later.

The study protocol was amended to assess possible effects of bFGF on lower extremity flow in the two high-dose groups using strain gauge plethysmography. At six months, the treated subjects in the 30 μg/kg once group had a near doubling of flow, while the 30 μg/kg twice group had a greater than three-fold increase over baseline lower extremity flow.

As physicians interested in the potential benefits of therapeutic angiogenesis, we were invited to comment on this report. We share two contrasting feelings. For several reasons, one feeling is very positive. First, this is a fascinating field of investigation that we hope will have a major beneficial impact on management of ischemic vascular disease. Second, this is an experienced group of investigators who have done excellent work on therapeutic angiogenesis in animal models of ischemia (5,8). They are now appropriately applying their expertise to clinical studies. Third, these initial safety, tolerability and pharmacokinetic data for bFGF in humans are reassuring. The authors present these data objectively and clearly state adverse effects without judgment. Fourth, the inclusion of a placebo control is a major strength.

On the other hand, this article causes us some misgivings, especially the estimates of lower extremity blood flow by venous occlusive plethysmography. First, the value of these results may be compromised because the number of patients is so small, and major differences exist between the placebo and treatment groups. The placebo group is older and has more subjects with hypertension and diabetes. Diabetics have a different distribution of peripheral arterial disease with more extensive calcifications (22). The treatment group had more peripheral vascular surgery done.

Second, the suggestion that bFGF causes a two- to three-fold increase in baseline flow is astonishing! Since this experiment has never been done, the results could be correct. However, the calf blood flow estimated by plethysmography is quite suspect (see comments to follow). One could argue that the authors need to be quite certain their data are correct and verifiable by additional estimates of blood flow before such extraordinary results are accepted.

Third, plethysmography may not adequately estimate lower extremity blood flow. Plethysmography has not been used for decades in outcome studies of peripheral vascular disease (22). Since the technique was developed for use in the forearm, the calculation of forearm volume may not apply to the leg with its different and more diverse shapes. Consistent venous occlusive flow measurements are not easy to obtain, and variability at best is $\pm 20\%$ between measurements. These concerns may be evident in the control group with an unexplained 67% increase in flow above baseline at one month, a value similar to the treatment group, only to return to near baseline at six months. Other techniques to support the finding from plethysmography could have been included. Ankle-brachial or toe systolic pressure index at rest and after exercise, total treadmill walk time and treadmill walk time to claudication are the most widely accepted measures of outcome in prospectively randomized placebo-controlled groups with arterial insufficiency of the lower extremities (22). Some of this information, available at baseline, unfortunately, was not reported after treatment.

CONCLUSIONS

Therapeutic angiogenesis has great potential for treating individuals suffering from tissue ischemia. More work is needed to realize that possibility. Safety, tolerability and pharmacokinetic studies of potential agents, such as the report of Lazarous et al. (21), are an essential first step in applying this technology to patients. On the other hand, publication of preliminary results purporting to show incredible benefit is not without scientific risk. Such reports often prove to be incorrect, due, in part, to data in a small number of subjects that are insufficient to support the claims. Therapeutic angiogenesis is an important area, certainly worthy of the highest quality scientific studies. The uniqueness of its potential should not alter that principle. We look forward to reading results of such studies from this group as well as others that have contributed so much to our understanding of angiogenesis.

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REFERENCES

1. Folkman J. Angiogenesis: initiation and control. Ann NY Acad Sci 1982;401:212–27.
2. Lewis BS, Flugelman MY, Weisz A, Keren-Tal I, Schaper W. Angiogenesis by gene therapy: a new horizon for myocardial revascularization? Cardiovasc Res 1997;35:490–7.
3. Folkman J. Angiogenic therapy of the human heart. Circulation 1998;97:628–9.
4. Ware JA, Simons M. Angiogenesis in ischemic heart disease. Nature Med 1997;3:158–64.
5. Kornowski R, Fuchs S, Leon M, Epstein SE. Delivery strategies to achieve therapeutic myocardial angiogenesis. Circulation 2000;101: 454–8.
6. Ferrara N, Davis-Smyth T. The biology of vascular endothelial growth factor. Endocrinol Rev 1997;18:4–25.
7. Hughes SE, Hall PA. Overview of the fibroblast growth factor and receptor families: complexity, functional diversity and implications for future cardiovascular research. Cardiovasc Res 1993;27:1199–203.
8. Unger EF, Banai S, Shou M, et al. Basic fibroblast growth factor enhances myocardial collateral flow in a canine model. Am J Physiol 1994;266:H1588–95.
9. Harada K, Friedman M, Lopez JJ, et al. Vascular endothelial growth factor administration in chronic myocardial ischemia. Am J Physiol 1996;270:H1791–802.
10. Baffour R, Berman J, Garb JL, Rhee SW, Kaufman J, Friedman P. Enhanced angiogenesis and growth of collaterals by in vivo administration of recombinant basic fibroblast growth factor in rabbit model of acute lower limb ischemia: dose-response effect of basic fibroblast growth factor. J Vasc Surg 1992;16:181–91.
11. Pu LQ, Sniderman AD, Brassard R, et al. Enhanced revascularization of the ischemic limb by angiogenic therapy. Circulation 1993;88:208–15.
12. Takeshita S, Pu LQ, Stein LA, et al. Intramuscular administration of vascular endothelial growth factor induces dose-dependent collateral artery augmentation in a rabbit model of chronic limb ischemia. Circulation 1994;90:II228–34.
13. Takeshita S, Zheng LP, Brogi E, et al. Therapeutic angiogenesis. A single intraarterial bolus of vascular endothelial growth factor augments revascularization in a rabbit ischemic hind limb model. J Clin Invest 1994;93:662–70.
14. Losordo DW, Vale P, Symes J, et al. Gene therapy for myocardial angiogenesis. Initial clinical results with direct myocardial injection of phVEGF$_{165}$ as sole therapy for myocardial ischemia. Circulation 1998;98:2800–4.
15. Rosengart TK, Lee LY, Patel SR, et al. Angiogenic gene therapy. Phase I assessment of direct intramyocardial administration of an adenovirus vector expressing VEGF121 cDNA to individuals with clinically significant severe coronary artery disease. Circulation 1999; 100:468–74.
16. Sellke FW, Laham RJ, Edelman ER, Pearlman JD, Simons M. Therapeutic angiogenesis with basic fibroblast growth factor: technique and early results. Ann Thor Surg 1998;65:1540–4.
17. Hendel RC, Henry TD, Rocha-Singh K, et al. Effect of intracoronary recombinant human vascular endothelial growth factor on myocardial perfusion. Evidence for a dose-dependent effect. Circulation 2000;101: 118–21.
18. Schumacher B, Pecher P, von Specht BU, Stegmann T. Induction of neoangiogenesis in ischemic myocardium by human growth factors. First clinical results of a new treatment of coronary heart disease. Circulation 1998;97:645–50.
19. Henry TD, Annes BH, Azrin MA, et al. Double-blind, placebo controlled trial of recombinant human vascular endothelial growth factor: the VIVA trial (abstr). J Am Coll Cardiol 1999;33 Suppl A:384A.
20. Baumgartner I, Pieczek A, Manor O, et al. Constitutive expression of phVEGF165 after intramuscular gene transfer collateral vessel development in patients with critical limb ischemia. Circulation 1998;97: 1114–23.
21. Lazarous DF, Unger EF, Epstein SE, et al. Basic fibroblast growth factor in patients with intermittent claudication. Results of a phase I trial. J Am Coll Cardiol 2000;36:1239–44.
22. Weitz JI, Byrne J, Clagett P, et al. Diagnosis and treatment of chronic arterial insufficiency of the lower extremities. A critical review. Circulation 1996;94:3026–9.