Delivery Strategies to Achieve Therapeutic Myocardial Angiogenesis

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Abstract—The use of recombinant genes or growth factors to enhance myocardial collateral blood vessel function may represent a new approach to the treatment of cardiovascular disease. Proof of concept has been demonstrated in animal models of myocardial ischemia, and clinical trials are underway. Currently, it is unknown which is the safest and most effective delivery strategy to induce clinically important therapeutic angiogenic responses in ischemic myocardium. Most strategies for transcatheter delivery of angiogenic factors have used an intracoronary route, which may have limitations because of imprecise localization of genes or proteins and systemic delivery to noncardiac tissue. The effect of direct intraoperative intramyocardial injection of angiogenic factors on collateral function has been reported in experimental models, and angiogenesis is being studied after direct intramyocardial injection of angiogenic peptides or plasmid vectors during open heart surgery in patients. Catheter-based transendocardial injection of angiogenic factors may provide equivalent benefit without the need for surgery. Intrapericardial delivery of angiogenic factors may offer a theoretical advantage of prolonged exposure of either coronary or myocardial tissue to the administered drug as result of a reservoir function of the pericardium. In this article, we review the different modes of administration for therapeutic myocardial angiogenesis therapy. (Circulation. 2000;101:454-458.)

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Animal studies have proved the feasibility of enhancing collateral function by delivery of angiogenic factors to the myocardium.1 This has been achieved either by intracoronary injection of the angiogenic factor at catheterization or by direct intramyocardial injection after thoracotomy.2–10 Successful angiogenesis has resulted from delivery of angiogenic proteins or plasmids or adenoviral vectors containing transgenes that encode angiogenic proteins. Currently, however, it is unknown which is the safest and most effective delivery strategy (ie, intracoronary versus intramyocardial injection versus intrapericardial delivery) to induce clinically important therapeutic angiogenic responses in ischemic myocardium.

Endovascular Administration

Data on the fate and efficacy of intravenous administration of angiogenic growth factors are limited. Intravenous bolus injection of basic fibroblast growth factor (bFGF) has been shown to be ineffective in inducing an angiogenic response in a canine model of myocardial ischemia11 in contrast to the efficacy of intracoronary administration achieved in the same model.3,4,5 This is likely due to “first-pass” uptake by low-affinity receptors in the lungs (consisting mainly of heparan sulfates, to which bFGF binds avidly), resulting in considerable lowering of the peak concentration delivered to the myocardium with intravenous compared with intracoronary administration. Because tissue uptake of bFGF is concentration dependent, the greater peak concentrations achieved at myocardial sites by intracoronary injection (unaffected by first-pass uptake by the lungs) would be expected to result in greater myocardial uptake than that achieved by intravenous injection.

This concept was proven by Lazarous et al,12 who evaluated differential regional uptake of 125I-labeled bFGF after bolus intravenous or via Swan Ganz, left atrial, intracoronary, or pericardial delivery in a dog model. After intracoronary administration, 3% to 5% of the bFGF dose was recovered from the heart, with the peptide found by immunohistochemical assessment to be localized to the extracellular matrix and vascular endothelium. In contrast, only 1.3% of the injected bFGF was localized to the heart after left atrial administration, and only 0.5% was recovered after intravenous or Swan Ganz delivery. Pericardial administration resulted in substantial cardiac bFGF delivery; 19% was present at 150 minutes. Myocardial uptake was similar with Swan Ganz and intravenous administration, suggesting that limiting delivery of the total drug dose to a pulmonary segment by Swan Ganz catheter, rather than the drug passing through the entire pulmonary bed as obtained with intravenous administration, still does not saturate available pulmonary binding sites. The importance of peak concentration in the determination of myocardial uptake is further emphasized by the observation that the serum half-life of bFGF was comparable after
intracoronary, intravenous, and left atrial delivery (≈50 minutes).

These data predict that myocardial angiogenesis will more likely be achieved after intracoronary, left atrial, or pericardial bFGF, whereas lack of efficacy would be predicted after intravenous and Swan Ganz administration. Similar data are lacking for vascular endothelial growth factor (VEGF) proteins. However, VEGF165, like the FGF family of peptides, is heparin binding. Thus, one would anticipate that peak concentrations delivered to the myocardium by intravenous administration would be limited in a manner similar to that seen with bFGF. In addition, maximal intravenous or intracoronary doses of both VEGFs and FGFs are limited by their propensity to cause hypotension.1,2,4,13,14

Intracoronary administration of an adenoviral vector expressing FGF-5 (Ad.FGF-5) and later Ad.FGF4 has been shown to improve function in ischemic myocardial regions of a porcine model of myocardial ischemia and appears to increase myocardial perfusion.3 An ≈95% first-pass myocardial uptake of the viral vector was reportedly achieved.3 This experimental study has led to an ongoing dose-escalating placebo-controlled clinical trial to test the safety, feasibility, and efficacy of intracoronary Ad.FGF-4 administration.

The effect of intracoronary administration of VEGF165 has been examined in 4 studies. Hariawala et al14 treated ischemic pigs with bolus injection of VEGF165 protein versus saline controls. They found that VEGF induced severe hypotension and/or shock, leading to mortality in 4 of 8 pigs in the high-dose (2 mg per pig) arm in their study. Thirty days after treatment, the surviving treated pigs and those injected with a lower dose of VEGF (500 μg per pig) had greater collateral perfusion than the untreated controls, suggesting a treatment effect. However, an alternative explanation for the results is that the surviving treated pigs survived the hypertensive effects of VEGF only because they had better collateral vessels, with the “improved” collateral function being the result of selection bias. Lopez et al15 compared 3 methods of intracoronary administration of VEGF165 protein in pigs with myocardial ischemia (20 μg per animal): periadventitial somatic pump delivery, intracoronary delivery with a local delivery catheter, or local intracoronary bolus injection. All 3 VEGF165-treated groups had a significant increase in perfusion and function assessments in the ischemic region.

In contrast, 2 other studies in a myocardial ischemic canine model suggested a less sanguine outlook for an angiogenic effect of a single intracoronary injection of VEGF. Thus, in an initial proof-of-concept study, Banai et al1 demonstrated that intracoronary VEGF administered daily for 28 days did enhance collateral function. However, in an attempt to gain insight into whether more clinically relevant administration strategies would also be effective, the same laboratory demonstrated that reducing the duration of intracoronary administration of VEGF to 7 days failed to enhance collateral function.

The first reported human clinical studies with intracoronary administration of VEGF165 protein were conducted to determine the appropriate dose and infusion rate of VEGF165 protein via the intracoronary and intravenous routes.16 In the subsequent randomized phase 2 study, patients were randomly assigned to 1 of 3 treatment groups (≈50 patients per group) and received a 10-minute intracoronary infusion of placebo or 17 or 50 ng·kg⁻¹·min⁻¹ VEGF165. This was followed by 3 sessions of 4 hours of intravenous infusions (placebo or 17 or 50 ng·kg⁻¹·min⁻¹ on days 3, 6, and 9). On day 60, the treated patients failed to meet the primary end point of improved exercise tolerance.16 In fact, not only did treated patients fail to meet the primary end point of improved exercise tolerance, but the patients receiving placebo and those received treatment improved their exercise tolerance to the same extent. Such a failure might have been anticipated given the conflicting animal data relating to brief intracoronary administration of VEGF and the data indicating severely impaired bFGF myocardial uptake after intravenous administration.

This study, although negative, is of critical importance to the field insofar as it puts into perspective the reliability of uncontrolled clinical trials. Its importance derives from the fact that it demonstrated unequivocally that a group receiving placebo treatment in a study designed to test the efficacy of angiogenic therapy exhibits just as much improvement as the “treated” group. The positive results achieved in a phase 1 clinical registry of recombinant bFGF administration must be interpreted with this in mind. bFGF was given via intracoronary route to 52 patients with coronary artery disease.17 The drug was administered as a single 20-minute infusion divided into 2 major sources of coronary blood supply. The escalated doses studied ranged from 0.33 to 48 μg/kg. At a 60-day follow-up, the angina class decreased from 2.6 to 1.2 (P<0.001), and exercise time increased from 8.5 to 10 minutes (P<0.05). Although nuclear perfusion imaging studies did not improve, MRI function and perfusion studies (which identify and quantify the myocardial region receiving delayed arrival of injected contrast) showed significant improvement. Overall, major adverse cardiac events (death or myocardial infarction) in this small study at 60 days was relatively high, occurring in 5 of 52 treated patients (~10%).

Potential Hazards

The primary concern with the pharmacological use of angiogenic growth factors has been the potential acceleration of occult neoplastic disease or retinopathy. Accordingly, safety end points directed toward examining those potential hazards should be included in myocardial angiogenesis trials.

From a cardiac viewpoint, the exposure of atherosclerotic plaques to angiogenic growth factors, such as occurs with intracoronary or intravenous administration, poses a potential hazard. The mitogenic effects of angiogenic growth factors may accelerate atherosclerotic lesion expansion and/or may lead to plaque destabilization, as demonstrated in several studies.18–20

To determine whether gene transfer to the vessel wall can succeed in producing biologic effects, Nabel et al18 used a eukaryotic expression vector to deliver the acidic FGF (aFGF) transgene into the coronary vessel wall of pigs. Three weeks after gene transfer, aFGF expression induced substantial intimal hyperplasia and intimal neocapillary formation compared with controls. In another animal study testing the
possible effects of VEGF on vascular lesions, the effects of VEGF on the neointimal response to acute vascular injury were assessed. The study used left atrial injections of VEGF protein daily for 7 days in a dog model of myocardial ischemia in which acute balloon-induced femoral arterial injury was produced. VEGF administration was associated with exaggerated neointimal thickening at the site of arterial injury.\textsuperscript{5}

In human studies, Flugelman et al\textsuperscript{9} found a strong association between smooth muscle cell proliferation, lesion “instability,” and the expression of aFGF and bFGF in human atherosclerotic plaques obtained by coronary atherectomy. More recently, Inoue et al\textsuperscript{30} described the expression of VEGF and its receptors in atherosclerotic lesions of human coronary artery, which were associated with the presence of inflammatory mononuclear cells within the plaques. Of probable relevance to this finding is the fact that VEGF is a chemoattractant for mononuclear cells.\textsuperscript{21,22}

Taken together, these data suggest that proatherogenic effects may exist as a consequence of intracoronary or intravenous administration of angiogenic growth factors. Whether this potential is only theoretical or whether it could lead to plaque expansion or instability that offsets any therapeutic angiogenic effects remains to be determined.

### Direct Intramyocardial Injections

There is a theoretical advantage of direct intramyocardial injection of angiogenic growth factors or angiogenic genes over intracoronary delivery. With intracoronary injection, a significant amount of the angiogenic factor will not be taken up from the vascular compartment by the heart during its first pass and therefore will be delivered to other tissues. It would thus appear desirable to deliver all the genetic material directly into the target tissue if possible.\textsuperscript{7,23}

This approach has been attempted successfully with an adenoviral vector containing the VEGF\textsubscript{165} transgene that was injected directly into ischemic myocardium of pig hearts during thoracotomy. Both myocardial perfusion and function in this model were significantly improved.\textsuperscript{8} The first clinical experience with intramyocardial injection of an angiogenic factor was recently reported.\textsuperscript{9} Recombinant human aFGF was injected directly into the myocardium just distal to the anastomotic site of the left internal mammary and anterior descending coronary artery.\textsuperscript{9} Twelve weeks later, angiographic studies were interpreted as being compatible with the formation of a collateral arterial network around the injection site. Additional preliminary uncontrolled clinical trials have used intramyocardial injection of naked DNA (VEGF\textsubscript{165} transgene) via a minimally invasive chest wall incision,\textsuperscript{10} Ad.VEGF\textsubscript{121} administered during conventional bypass surgery, and Ad.VEGF\textsubscript{121} administered as sole therapy.\textsuperscript{24} Preliminary findings demonstrated the procedural safety and feasibility this proangiogenic approach.

Although of potential clinical importance, the practical application of the transthoracic transepicardial strategy for therapeutic angiogenesis is clearly limited because of the risks and expenses associated with the invasive nature of the procedure. Catheter-based transcendocardial injection of angiogenic factors may provide equivalent benefit without surgery. Recently, the feasibility of various catheter-based systems for catheter-based intramyocardial injection of marker genes has been demonstrated in animal models.\textsuperscript{25,26}

It has recently been demonstrated that using a percutaneous magnetic field–guided approach for intramyocardial injection allows delivery of gene products into prespecified treatment zones.\textsuperscript{26,27} This has been achieved despite endocardial motion and without perforation or other undesirable tissue effects or serious ventricular arrhythmias. It remains to be determined whether comparison of the transcendocardial and transepicardial modes of administration would result in a similar efficiency of gene delivery, protein expression, and proangiogenic response.

### Intrapericardial and Perivascular Delivery

Another mode of delivery for therapeutic myocardial angiogenesis has been the use of drug delivery into the pericardial sac.\textsuperscript{28–31} This mode of administration offers a theoretical advantage of prolonged exposure of either coronary or myocardial tissue to the administered drug resulting from the reservoir function of the pericardium. Although several animal studies have suggested that intrapericardial administration of either bFGF\textsuperscript{28,29} or aFGF\textsuperscript{30} improves regional myocardial blood flow and function while causing myocardial vessel proliferation, other studies using bFGF protein have failed to show benefit.\textsuperscript{32} Although animal studies have supported the feasibility and safety of the intrapericardial delivery approach incorporating a sheathed needle with a suction attachment to grasp the pericardium,\textsuperscript{23} even if this route of delivery proves efficacious, it is questionable whether such a delivery mode will be feasible and practical in patients with multiple prior instrumentation, including previous coronary artery bypass surgery.

Prolonged exposure to angiogenic peptides can also be achieved by direct epicardial deployment of sustained-release polymers for extravascular elution of angiogenic growth factors over time. The first randomized controlled surgical experience with perivascular administration of bFGF (10 or 100 \textmu g) using heparin-alginate microcapsules implanted at the subepicardial fat have proved the safety and feasibility of this approach in patients undergoing coronary bypass surgery.\textsuperscript{34} It remains to be determined whether such adjunctive angiogenic pharmacotherapy to coronary bypass surgery will improve the long-term outcomes of surgically treated patients.

### Measuring the Efficacy of Angiogenic Intervention

A common problem in all delivery modalities is measuring the efficacy of proangiogenic interventions. Anginal symptoms and quality-of-life evaluations are too subjective and should not be used as sole efficacy end points. Surrogate end points to clinical evaluation, such as exercise tests, nuclear perfusion scans, stress echocardiography (with or without contrast), and angiographic collateral assessments, have been difficult to quantify and may not be sufficiently sensitive to detect subtle changes in myocardial perfusion or function after angiogenic interventions. PET studies\textsuperscript{35} and more recently MRI perfusion imaging\textsuperscript{36} have been used to assess
myocardial flow, metabolism, and/or function. Those modalities, albeit not yet widely spread, may be more sensitive than conventional imaging techniques in assessing the effect of proangiogenic interventions among patients.

Conclusions

The use of recombinant genes or growth factors to enhance myocardial collateral blood vessel function may represent a new approach to the treatment of ischemic cardiovascular disease. Most strategies for transcatheter delivery of angiogenic factors have used an intracoronary route, which has theoretical limitations because of imprecise localization of genes or proteins and systemic delivery to noncardiac tissue. The effect of direct intraoperative intramyocardial injection of angiogenic factors on enhancing collateral function has been demonstrated in experimental models. Although the transseptal delivery of angiogenic factors in humans at the time of surgery has been performed, it appears likely that catheter-based transcatheter injection of angiogenic factors can achieve equivalent efficacy in terms of delivering therapeutic agents into the myocardium. However, much is still unknown about the optimal delivery of angiogenic factors, including the critical issue of whether angiogenesis therapy works in humans. Assuming that it does, we still do not know which is the best region to deliver the factor. Should it be delivered directly into the ischemic region, to the peri-ischemic or nonischemic regions from which the collaterals will originate, or to both? If the last option is the case, intracoronary delivery may become a practical strategy, assuming that the therapeutic dose can be safely delivered via this route. Such an outcome would also mean that sophisticated catheter-based delivery systems that allow for precise localization for delivery of therapeutic agents will be unnecessary. On the other hand, if targeted therapy proves to be safer and either more or equally efficacious, guided transcatheter delivery may be optimal.

The only way to answer these questions is through controlled clinical trials. Fortunately, many trials either are currently underway or will be initiated soon. It is to be hoped that in the not-too-distant future, these investigative efforts will lead to critically important new strategies for treating patients with myocardial ischemic syndromes.

References

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