Surgical and percutaneous myocardial angiogenesis induction

Part II - Neoangiogenesis

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Coronary artery bypass surgery and angioplasty provide symptomatic relief in patients with ischemic heart disease, but despite advancement in technique and devices, these methods are not applicable to a subset of patients with angina refractory to medical treatment. Bypass surgery might not be feasible because of lack of suitable conduits, diffuse coronary disease or poor distal run-off, and coronary angioplasty is sometimes not applicable due to chronic total occlusion, diffuse disease or extreme tortuosity.

We have previously reviewed the available experience with laser-induced direct myocardial revascularization, one of the new potential treatment modalities for this patient subset. One of the potential mechanisms of action for laser treatment is the induction of neoangiogenesis. In the second part of our article we review the available experience with the induction of myocardial angiogenesis using different growth factors or the genes encoding for them.

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Introduction

Coronary artery bypass surgery and angioplasty cannot offer complete revascularization in a subset of patients with diffuse coronary artery disease with poor distal runoff or extreme vessel tortuosity. We previously reviewed the possible mechanisms by which direct myocardial revascularization can improve symptoms in patients with angina pectoris refractory to treatment, namely placebo effect, denervation, or the induction of neoangiogenesis.

For patients who are considered poor candidates for surgery and angioplasty, another promising method currently under investigation is the induction of neoangiogenesis by growth factors. Several growth factor proteins or the genes encoding for them have been shown to induce neoangiogenesis, and can be administered by direct intramyocardial injection, during thoracotomy, by catheter-based technique or by injection into the coronary arteries or peripheral veins.

The turnover rate of the cells of normal capillaries is very low, ranging from months to years. Occasionally, in certain physiological conditions like development of placenta, fetal growth, wound healing and development of collaterals in response to tissue ischemia, the turnover rate of these cells increases significantly. Angiogenic growth factors catalyze this process by increasing cell proliferation, differentiation and migration via the specific receptors on the surface of the vascular endothelial cells[1-4]. The reduced angiogenic competence in older patients and females with the resultant higher complication rate in this population following myocardial infarction can be attributed to locally decreased angiogenic growth factors[5]. In 1985, Gimenez-Gallego et al.[6] elucidated the biochemical structure of fibroblast growth factor-1 (FGF-1). Human FGF-1 was isolated from brain tissue in 1986[7]. FGF-1 was successfully expressed in apathogenic strains of *Escherichia coli* transfected using the technique of gene transfer[8]. The angiogenic effect of vascular endothelial growth factor (VEGF) is mediated by a synergistic action of nitric oxide and prostacyclin[9].

In a rabbit model, intracoronary administration of growth factor (FGF) after liga-

tion of a coronary artery induced notable development of collaterals and reduction in infarct size[10]. Baffour et al.[11] reported a significant formation of collaterals in the ischemic extremities of animals following treatment with FGF. Similarly, Albes et al.[12] successfully improved blood flow in ischemic tracheal segments implanted subcutaneously in rabbits with local injection of FGF enriched fibrin glue. The ability of FGF to potentiate cell proliferation can be augmented by adding heparin which protects growth factor from denaturation by proteolytic enzymes[13]. Several other preclinical animal studies have confirmed the ability of growth factors to induce angiogenesis in the lower limbs[14] as well as in the myocardium[15-17].

After preliminary studies of the effects of FGF-1 in ischemic rat hearts, Schumacher et al.[18], in a landmark clinical trial, injected 0.01 mg/kg body weight of FGF-1 in 20 patients with triple vessel disease close to the internal mammary artery to the left anterior descending artery anastomosis. All the patients had additional peripheral stenoses of the left anterior descending artery or one of its diagonal branches. At 12 weeks, the internal mammary artery bypasses were selectively imaged by intra-arterial digital subtraction angiography and quantitatively evaluated. In all the patients, a capillary network sprouting from the proximal part of the coronary artery could be shown to have bypassed the stenosis and rejoined the distal part of the vessel with a resultant 2 to 3-fold increase in the local blood supply.

As an alternative to direct intramyocardial injection of FGF, Laham et al.[19] tested the efficacy of intracoronary administration of recombinant FGF-2 in a phase 1 trial involving 52 patients. They reported that this route was safe over a wide range of doses and resulted in decreased angina frequency, increased exercise tolerance, and improved regional left ventricular function and perfusion as assessed by magnetic resonance imaging. In a randomized placebo-controlled trial by Laham et al.[20], 24 patients were randomized to 10 μg of bFGF (8 patients), 100 μg of bFGF (8 patients) and placebo (8 patients), in addition to undergoing coronary bypass surgery. There were two operative deaths and three Q wave myocardial infarctions. There were no treatment-related adverse cardiac events, and there was no rise in serum bFGF levels. Three control patients had recurrent angina, 2 of whom required repeat revascularization. One patient in the 10 μg group had angina, whereas all patients in the 100 μg bFGF group remained angina-free. Stress nuclear perfusion imaging at baseline and 3 months after bypass surgery showed a trend towards worsening of the defect size in the placebo group, no significant change in the 10 μg group, and a significant improvement in the 100 μg group. Magnetic resonance assessment of the target ischemic zone in a subset of patients showed a trend towards a reduction in the target ischemic area in the 100 μg group.

Human VEGF also known as vascular permeability factor or vasculotropin was first isolated in 1983[21]. It is an important angiogenic factor that induces migration and proliferation of endothelial cells, enhances vascular permeability and modulates thrombogenicity. VEGF has a role in tumor growth, wound healing, age-related macular degeneration, rheumatoid arthritis, diabetic nephropathy, and growth of collaterals in ischemic tissue[22]. The major advantage of VEGF is that it is specifically mitogenic for endothelial cells which represent the critical cellular element responsible for neoangiogenesis. On administration of recombinant human VEGF (rhVEGF), angiographically apparent collaterals arise both directly from the parent epicardial artery and from existing collaterals[23]. Baumgartner et al.[24] have recently concluded a study of angiogenesis using gene transfer in the treatment of critical limb ischemia. They administered 4000 μg of naked plasmid DNA encoding VEGF to each of the 10 ischemic limbs of 9 patients by direct intramuscular injection. Transient elevation in serum levels of VEGF confirmed gene expression in these limbs. At follow-up, they noted resolution of ischemic ulcers in 4/7 limbs, development of new collateral blood vessels as documented by contrast angiography in 7/10 limbs and improved distal flow in 8/10 limbs on magnetic resonance angiography. Successful limb salvage was achieved in 3 patients recommended for below-knee amputation.

A subsequent report by Losordo et al.[25] suggested that direct myocardial injection of naked plasmid DNA via a minimally invasive chest wall incision is safe, can reduce the symptoms and improve myocardial perfusion in selected patients with chronic myocardial ischemia. In 5 patients with refractory class III or class IV angina, naked plasmid DNA encoding $_{rh}VEGF_{165}$ was injected into the ischemic myocardium via a mini left anterior thoracotomy. Objective evidence of reduced ischemia was documented using dobutamine single photon emission computed tomography (SPECT)-sestamibi imaging in all patients. Coronary angiography showed improved Rentrop score in all patients.

The results of the first randomized placebo-controlled trial of rhVEGF to assess the safety and efficacy of this form of treatment have recently been presented[26]. This study included 178 patients with stable angina who were not optimal candidates for coronary angioplasty or bypass surgery but had a significant reversible defect by SPECT nuclear perfusion studies. RhVEGF 17 or 50 ng/kg/min protein or placebo was administered by two 10-min intracoronary infusions on day 0, followed by three 4-hour i.v. infusions of placebo or 17 and 50 ng/kg/min of rhVEGF on day 3, 6 and 9. RhVEGF protein was safe and well tolerated. Improvements in treadmill time did not differ significantly in the two groups, but there was a trend towards improvement in angina class in the rhVEGF treated patients. Possible explanations for the absence of clinical benefit include the route of administration and the protein dose.

Inoue et al.[27] have provided evidence of the role played by VEGF in the progression of human coronary atherosclerosis as well as in recanalization processes in obstructive coronary disease. The significance of this potentially worrisome observation needs further evaluation. There are also some other reasons for caution which need to be clarified with further research. It is possible that VEGF expression resulting from gene transfer could promote the development of a tumor that is too small to be recognized at this early stage of clinical trials. Studies by Ferrara et al.[28] however have shown that although VEGF expression promotes growth process, it does not lead to malignant proliferation or metastasis. VEGF could aggravate deteriorating eyesight due to diabetic retinopathy and age-related maculopathy. Mild hypotension has been observed in animal studies following growth factor administration[15,29]. It could occur even in clinical studies and is a potential complication that requires close observation. It also has a possible role in the progression of atherosclerosis and may prove to be a double-edged sword.

Systemic delivery of genetic material and its potential drawbacks could potentially be avoided with a strategy of direct intramyocardial injection. Furthermore, a catheter-based intramyocardial injection system could provide more site-accurate injection of genes or peptides without surgery or general anesthesia. Kornowski et al.[30] tested the feasibility of a new guiding system (NOGA BioSense, Johnson & Johnson, Tirat, Hacarnel, Israel) utilizing low-intensity magnetic field energy and a sensor-tipped catheter integrated with a 27G needle to inject the gene encoding adenovirus VEGF 121 in the porcine myocardium. The potential advantages of such an approach are the precise localization of the injection sites, thus avoiding same-site injections, and the decreased fluoroscopy time. In their study, the high levels of VEGF 121 production obtained was of similar magnitude whether injected using the transendocardial or the transepicardial delivery approach. Interestingly, there was a significant drop-off of VEGF production at a short distance from the injection site. During injection there were no serious complications such as death, destabilizing arrhythmias or cardiac tamponade. Only in 5-10% of the injections was any trace of the genetic material found, thus raising the possibility of systemic delivery.

Conclusions

Currently it is unknown which is the most effective and safe delivery strategy to induce therapeutic angiogenesis at the myocardial level.

The availability of percutaneous delivery systems and the guidance obtained with non-fluoroscopic three-dimensional mapping of the left ventricle offer a powerful tool for direct injection of angiogenic factors in the myocardial segments of interest. This represents a potentially useful approach, but the biologic and clinical importance of angiogenic treatment has yet to be ultimately proven.

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