

Therapeutic Myocardial Angiogenesis Using Percutaneous Intrapericardial Drug Delivery

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Summary: In this manuscript, we describe the potential role of the pericardial space as a drug delivery reservoir to administer angiogenic agents to the heart resulting in functionally significant angiogenesis with single bolus basic fibroblast growth factor (bFGF) delivery. We also describe a percutaneous subxyphoid pericardial access technique that is safe, rapid, and reliable.

Key words: ischemic heart disease, angiogenesis, fibroblast growth factor, pericardium

Introduction

Ischemic heart disease remains the leading cause of mortality and morbidity in the Western hemisphere. Therapeutic approaches to the management of chronic myocardial ischemia traditionally include efforts aimed at reducing the progression of coronary disease (risk factor modification and aggressive lipid-lowering strategies), reducing myocardial oxygen demand, and preventing cardiac events (medical therapy using antiplatelet agents, beta blockers, angiotensin-converting enzyme inhibitors, and calcium-channel blockers), or increasing blood supply to compromised territories by providing new [coronary artery bypass surgery (CABG)] or restoring old [percutaneous transluminal coronary angioplasty (PTCA)]

pathways for blood flow. However, it is becoming clear that a significant proportion of patients with ischemic heart disease are suboptimal candidates for CABG/PTCA and are refractory to medical therapy.¹⁻⁵ An alternative to these approaches may include an attempt of inducing growth and development of new collateral vessels (therapeutic angiogenesis). Angiogenesis is a complex process involving endothelial cell proliferation and migration, formation of new capillaries, attraction of pericytes and macrophages, stimulation of smooth muscle cell proliferation and migration, breakdown of existing extracellular matrix, formation of new vascular structures, and deposition of new matrix.⁶⁻⁸

The increased expression of various heparin-binding growth factors and their receptors in ischemic myocardium and the ability of these cytokines to induce endothelial cell proliferation and migration *in vitro* has led to their rapid development for therapeutic angiogenesis for myocardial and peripheral limb ischemia.⁶⁻¹⁴

Therapeutic Angiogenesis

We and others have shown that various heparin-binding growth factors including basic fibroblast growth factor (bFGF),¹⁵⁻²¹ acidic FGF (FGF-1),²² and vascular endothelial growth factor (VEGF)^{15, 23-25} induce angiogenesis in chronic myocardial ischemia.

Daily injections of 110 µg of bFGF for 18 days directly into the circumflex coronary artery distal to an ameroid occluder hastened restoration of flow in the compromised territory compared with normal saline controls.¹⁹ Morphometric analysis of left circumflex (LCx) myocardium demonstrated a significant (2-fold) increase in the number of larger (>20 µm) vessels.¹⁹ Daily left atrial injections of 1.74 mg of bFGF for 18 days in the same animal model resulted in early augmentation of coronary flow in the growth factor-treated animals that was comparable with that seen with direct intracoronary injections but was lost by day 38.²⁶ In the same canine model, 7-day systemic arterial administration of bFGF enhanced collateral development without increasing neointimal accumulation at sites of vascular injury.¹⁵ Local perivascular delivery of bFGF

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was evaluated in a porcine model of chronic myocardial ischemia (LCx ameroid occlusion). Heparin-alginate microcapsules were used for sustained delivery of bFGF. Animals implanted with heparin-alginate pellets containing 8 μg of bFGF at the time of ameroid placement demonstrated significantly better preservation of perfusion of the ischemic zone during pacing compared with control animals.¹⁷ In addition, ventricular function studies demonstrated better preservation of regional left ventricular function in the ameroid-compromised territory at rest and faster recovery following pacing in bFGF-treated animals.¹⁷ Examination of the effect of progressively larger amounts of bFGF (10 and 100 μg), delivered in a similar manner in a pig model,²⁷ demonstrated substantial improvement in resting coronary blood flow in the chronically ischemic myocardium in both bFGF groups compared with controls and an increase in angiographic collaterals.^{17, 27} Analysis of left ventricular function demonstrated a higher ejection fraction at rest and during pacing in both 10 and 100 μg bFGF groups compared with controls.

Treatment with VEGF also results in a myocardial angiogenic response in animal myocardial ischemia models. A study carried out in a dog ameroid model suggested that daily intracoronary injections of 45 μg of VEGF delivered distal to the occluder over a 28 day period (total dose 900 μg) result in faster restoration of collateral zone flow than did similar injections of normal saline.²⁵ Morphologic analysis demonstrated a significantly higher number of small vessels in VEGF-treated compared with control animals. The therapeutic efficacy of VEGF in porcine circulation was tested using an implantable minipump primed with 2 μg of VEGF and 50 U of heparin delivered over 4 weeks periadventitally to the circumflex coronary artery distal to the ameroid occluder.²³ Comparison of VEGF/heparin- and heparin only-treated animals demonstrated that while coronary flow in the ischemic territory at rest was no different between the two groups, VEGF treatment was associated with better coronary flow during pacing. Assessment of myocardial perfusion using magnetic resonance imaging demonstrated not only significantly better perfusion of the compromised territory in VEGF-treated animals but also a reduction in the size of this territory.²⁴ Morphometric analysis found a nearly 4-fold increase in the number of collateral vessels in VEGF-treated animals that was limited to the ischemic zone.²³ Analysis of microvascular function demonstrated significantly better restoration of endothelium-mediated, receptor-dependent relaxation in VEGF-treated animals.^{23, 28} These improvements in coronary flow and microvascular function were reflected in enhanced left ventricular performance in VEGF-treated animals, as demonstrated by higher ejection fraction and better preservation of regional wall shortening during pacing stress.²³

The efficacy of VEGF (20 μg) single bolus intracoronary injection was compared with the same amount of VEGF delivered either perivascularly or locally using an InfusaSleeve™ catheter (LocalMed, Inc., Palo Alto, Calif., USA).^{28, 29} The studies conducted in a porcine ameroid constrictor model demonstrated that, compared with control animals, both intracoronary bolus injection and local delivery resulted in sign-

ificant increase in angiographically detected left-to-left collaterals and improvement in myocardial blood flow, regional left ventricular function, and microvascular function.

Given the typically long time course of new collateral vessel development, most attempts to stimulate myocardial angiogenesis have employed methods of prolonged growth factor delivery, including gene therapy, continuous infusions, repeated injections, or sustained release polymers.^{15–19, 23, 25, 30} However, some of these options are either unfeasible or impractical in patients with ischemic heart disease, making single-dose administration, if effective, a potentially superior strategy in these patients.

The Role of the Pericardium

The pericardium likely plays an important role in the regulation of several myocardial processes. The concentration of bFGF and VEGF in the pericardial fluid of patients with unstable angina is significantly higher than in patients with nonischemic heart disease.³¹ The concentration of bFGF in pericardial fluids in ischemic patients was 2036 ± 357 pg/ml, significantly ($p < 0.001$) higher than 289 ± 72 pg/ml in nonischemic patients. The concentration of VEGF in the pericardial fluid tended to be higher in ischemic patients, but the difference was not statistically significant (39 ± 7 vs. 22 ± 6 pg/ml). The increased levels of such endogenous proangiogenic growth factors in the pericardial fluid of patients with ischemic heart disease suggest a physiologic role for these factors in the host response to myocardial ischemia and injury. This observation coupled with the favorable pharmacokinetic profile of some drugs in the pericardial space suggests that the pericardial space may potentially serve as a unique drug delivery reservoir for the delivery of therapeutic agents to the heart.

Therapeutic Angiogenesis Using Pericardial Delivery

Landau *et al.* studied the effects of an intrapericardial bFGF infusion in a model of chronic myocardial ischemia. Intravenous angiotensin II (AII) was infused to induce left ventricular hypertrophy and concomitant ischemia in New Zealand white rabbits.¹⁸ Basic fibroblast growth factor was infused into the intrapericardial space using an osmotic pump. Epicardial angiogenesis was graded histologically on a scale of 0 to 2. Animals receiving intravenous AII displayed left ventricular hypertrophy. A highly localized angiogenic effect of bFGF was observed compared with control animals.¹⁸ Uchida *et al.* studied the effect of intrapericardial bFGF (30 μg bFGF + 3 mg heparin) in a canine model of acute myocardial infarction. One month later infarcted weight/left ventricle weight was $24 \pm 5.2\%$, 25 ± 4.0 , 18 ± 2.4 , and $10 \pm 1.8\%$ with saline, heparin, bFGF alone, and bFGF + heparin administration, respectively.²⁰ Vascular number in the infarcted area of the outer layer was the largest in the bFGF + heparin group (13 ± 3.3 , 20 ± 2.2 , 47 ± 8.3 , and 136 ± 26.3 in the saline, heparin, bFGF alone, and bFGF + heparin groups, respectively). The vascular

number was larger in the subepicardial than in the subendocardial infarcted areas.²⁰

Thus, intrapericardial administration of growth factors results in histologic evidence of neovascularization^{18,20} and a reduction in myocardial infarction extent.²⁰ The infusion of growth factors in these studies and the hypertrophy model and acute myocardial infarction model in these studies limits their applicability to patients with ischemic heart disease secondary to progressive atherosclerosis. Therefore, a single dose intrapericardial administration of angiogenic growth factors in a chronic myocardial ischemia model was needed utilizing the pericardium as a drug delivery reservoir to deposit these therapeutic agents.

Therapeutic Angiogenesis Using Single-Dose Intrapericardial Administration of bFGF

We have recently completed an animal study of intrapericardial bFGF delivery for therapeutic angiogenesis in a porcine chronic myocardial ischemia model, using a percutaneous subxyphoid pericardial access technique.^{32–34} Forty-nine Yorkshire pigs underwent ameroid placement on the LCx artery. Three weeks after ameroid placement, animals underwent coronary angiography to confirm LCx occlusion and to assess the extent of angiographic collaterals. After microsphere injection and determination of regional left ventricular function, myocardial perfusion, and collateral extent using magnetic resonance imaging,^{24,35} the animals underwent percutaneous (nonsurgical) subxyphoid access of the pericardial space using a blunt-tipped needle under fluoroscopic guidance.³² Access of the pericardial space was confirmed by the injection of 1 ml of diluted contrast.³² Then, the animals were randomized to receive bFGF (30 µg, 200 µg, or 2 mg) or saline/heparin. Four weeks later, the animals underwent repeat angiography, left atrial microsphere injection, magnetic resonance imaging for regional left ventricular function, myocardial perfusion, and collateral extent. They were then sacrificed and the hearts were excised for morphometric analysis, microsphere blood flow determination, and measurement of microvascular function to determine endothelial-dependent and -independent vasodilation.^{28,33,36–38}

Percutaneous subxyphoid access of the pericardium was successful in all animals without any complications,³² and drug delivery was accomplished in all cases. Three weeks after implantation of ameroid occluders, at the time of intrapericardial drug delivery, myocardial vascular resistance in the collateral-dependent LCx territory was similar in all treatment groups and was significantly higher than resistance in the left anterior descending (LAD) territory. Four weeks following intrapericardial drug delivery, LCx resistance was significantly lower in bFGF-treated animals than in controls,³³ and bFGF treatment resulted in a significant increase in angiographic collaterals, regional myocardial blood flow, myocardial function in the ischemic territory, collateral extent, and myocardial vascularity compared with control animals.³⁴ Microvascular analysis showed that endothelium-dependent vasodilation was normal in the LAD but not in the LCx distri-

bution in control animals, indicating dysfunctional endothelium in the ischemic zone; however, bFGF treatment improved endothelium-dependent vasodilation in the LCx epicardium.³³ Thus a single bolus administration of bFGF in a porcine model of chronic myocardial ischemia resulted in functionally significant angiogenesis.

Conclusion

The pericardial space appears to play an important role in the physiologic and pathologic regulation of various myocardial processes. The elevated levels of various angiogenic cytokines in patients with myocardial ischemia indicate a potential role of the pericardium in ischemia-induced angiogenesis. Intrapericardial or epicardial administration of these cytokines appear to result in functionally significant angiogenesis in animal models of chronic myocardial ischemia. The above-described study demonstrated the ability of a single bolus injection of bFGF into the pericardial space to induce myocardial angiogenesis and improve regional perfusion, regional left ventricular function, and microvascular reactivity, and to increase angiographically visible collaterals and magnetic resonance-detected collaterals. Thus, the pericardial space offers an attractive drug delivery reservoir that might be used to deliver therapeutic substances to the heart.

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