Review

Angiogenic gene therapy for heart disease: a review of animal studies and clinical trials

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Abstract

The current published clinical literature on angiogenic gene therapy for the treatment of myocardial ischemia does not include a single randomized, placebo-controlled trial. Based on current clinical literature, it is an unproven therapy. Successful animal studies combined with published reports of good outcomes in patients enrolled in uncontrolled trials has led to the expectation that angiogenic gene therapy will ultimately become a clinical reality. The next important landmark in the field will be the publication of data showing a favorable effect of angiogenic gene transfer in placebo-controlled, blinded clinical trials. © 2001 Elsevier Science B.V. All rights reserved.

1. Introduction

Studies exploring the efficacy and safety of angiogenic gene therapy for the treatment of heart disease using animal models have appeared with increasing frequency since first reported in 1996 [1] and preliminary uncontrolled clinical trials recently have been published. For extensively referenced reviews of angiogenesis the reader need not look far — such reviews recently have been proliferating. The current review is, by design, more limited in scope. Specifically, we will (with a single exception) examine peer-reviewed original publications (to October 2000) regarding angiogenic gene transfer in experimental or clinical heart disease. The publications that fulfill these criteria are few in number. Abstracts are not reviewed.

Because of the absence of published placebo-controlled randomized clinical studies, we decided to review all trials that met the above criteria regardless of whether the studies included appropriate controls. Obviously placebo-controlled trials will be required before definitive conclusions can be drawn. We have reviewed all studies of angiogenic gene transfer in experimental or clinical heart disease, whether the method of delivery was thoracotomy and intramuscular injection into heart muscle (many), nonsurgical intracoronary delivery (few), or injection into the pericardial space (one).

We divide the review into four sections — intramuscular injection of adenovirus vectors; intramuscular injection of plasmid DNA; injection of adenovirus into the pericardial space; and intracoronary delivery of adenovirus. Within each section we divide the papers into those performed in animal models of heart diseases and clinical trials. Within the subgroups we generally have reviewed papers in chronological order from the earliest published papers to the most recent. When appropriate we have included comments regarding strengths and weaknesses of each publication.

Both authors have ties to Collateral Therapeutics, Incorporated (CTI) a San Diego biotechnology company focused on nonsurgical treatment of cardiovascular diseases using gene transfer. Dr. McKirnan, who worked with animal models of cardiovascular diseases at the University of California San Diego (UCSD) for over 20 years, is now Associate Director of Research at CTI. Dr. Hammond, a Professor at UCSD, the scientific founder of CTI serves as a consultant and has a proprietary interest in the company.

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These associations were disclosed when the editors asked that a review be written, a request that was initially declined. However, the editors desired participation despite these associations. We have maintained a critical spirit throughout the review and have attempted to be fair and impartial. We are hopeful that our colleagues will pardon our critical comments and that we have been equally critical of the few papers coming from our own laboratory. We apologize if we failed to review any studies that fulfilled criteria for review.

2. Intramyocardial injection of adenovirus using thoracotomy

2.1. Animal studies

A report of the potential usefulness of direct intramyocardial injection of an adenovirus encoding an angiogenic gene in the setting of experimentally induced myocardial ischemia was contained in a paper by Rosengart and Crystal and colleagues at Cornell [2]. They used the porcine ameroid model of stress-induced regional myocardial ischemia to determine whether intramyocardial injection of an adenovirus encoding vascular endothelial growth factor-121 (Ad.VEGF-121) would reduce stress-induced regional myocardial ischemia. Regional function (echocardiography) and perfusion (radionuclide imaging) were assessed 3 weeks after proximal left circumflex ameroid placement. Then, at a second thoracotomy, Ad.VEGF-121 or an empty adenovirus vector was injected by needle insertion into the wall of the heart — ten injections in the left circumflex perfusion bed each consisting of 10^8 plaque forming units (pfu) — and studies of regional function and perfusion were repeated 4 weeks later. The relationship between virus particle number (vp) and pfu (a measure of infectivity) is dependent on the specific adenovirus and the assays used, but generally is around 100 vp per 1 pfu. Therefore, this amount of adenovirus (10^8 pfu) would be roughly comparable to 10^10 vp. Both regional function and perfusion during stress were improved in Ad.VEGF-121 treated animals.

This study used the best animal model of regional stress-induced myocardial ischemia and employed suitable methods to assess regional function and perfusion. It is impressive that the degree of gene transfer conferred by such relatively low amounts of adenovirus resulted in such improvements. A paper examining intramuscular injection of adenovirus in porcine myocardium found limited amount of reporter gene activity seen predominantly adjacent to the needle tract [3]. Although the method of gene transfer used by Rosengart, Crystal and colleagues required thoracotomy, the results were impressive. It is not clear that the acquisition of data was blinded, an important factor to increase the value of echocardiographic studies. However, data analyses were conducted in a blinded manner.

A second paper, also from the same group, used the porcine ameroid model of stress-induced myocardial ischemia to determine what toxic effects might result from intramyocardial injection of Ad.VEGF-121 [4]. Twenty-one days after left circumflex ameroid placement a second thoracotomy was performed and ten sites in the left circumflex perfusion bed were injected. Doses per injection site were 10^8 and 10^9 pfu. In addition, replication competent adenovirus and replication incompetent adenovirus encoding no transgene were also injected (10^9 pfu per site, ten sites). It should be pointed out that replication competent adenovirus would not likely replicate in porcine cells as the vector used was a human adenovirus. Echocardiographic assessment, survival, blood analysis, and myocardial and liver histology were examined 3 and 28 days after vector administration. Inflammation and necrosis (described as minimal) were observed in hearts of animals that had received Ad.VEGF-121 gene transfer, but livers were normal. The Ad.VEGF-121 treated animals also showed an elevation in white blood cell count 3 days after treatment (vs. other groups) that was mild and transient.

The echocardiograms were apparently obtained only to determine if there was an adverse effect of vector administration on basal regional function (no deleterious effects were seen) — no data regarding regional myocardial function during stress were reported. The myocardial inflammation and necrosis observed, while mild, was statistically significant and dose-related, with increased amounts in the animals that had received 10^9 pfu per site. This amount of adenovirus is 75% less than the dose used that was associated with marked inflammation reported by French et al. [3].

Departing for the moment from the porcine ameroid model of stress-induced reversible myocardial ischemia, what effect might angiogenic gene transfer have in the setting of acute coronary occlusion? Studies from Capogrossi’s group at the National Institutes of Health tested two hypotheses — first, that angiogenic gene transfer results in angiogenesis even when ischemia is not present, and second, that subsequent acute coronary artery occlusion is associated with a reduced bed at risk after angiogenic gene transfer [5]. Rabbits underwent thoracotomy and intramyocardial injection of 10^9 pfu adenovirus encoding lacZ (control) or acidic fibroblast growth factor (aFGF, also known as FGF-1) with an engineered signal sequence to promote extracellular secretion of the transgene protein. The adenovirus vector was injected in the myocardium adjacent to the coronary artery that would, 12 days later, be occluded and the size of the perfusion bed assessed. They found a 50% reduction in the perfusion bed at risk and evidence for angiogenesis and vasculogenesis in hearts of animals that had received Ad.FGF-1 gene transfer. Specifically, there was a two-fold increase in length-density of intramural arterioles and a 17% increase in the capillary network.

The study demonstrates the potential usefulness of myocardial FGF overexpression, however, the model is not
directly applicable to clinical settings. That is, it is unlikely that thoracotomy and intramyocardial gene transfer of Ad.PGF-1 would be considered in anticipation of myocardial infarction. Even so, this was a compelling study that adds to the body of experimental data indicating that overexpression of FGF can have a favorable impact in experimental models of coronary artery disease. The methods used to assess angiogenesis and vasculogenesis are superior to most of the studies so far reported in the field of therapeutic myocardial angiogenesis in that they included appropriate controls, used perfusion-fixed samples, and included a two-dimensional analysis. In addition, these studies were blinded, a critical element because microscopic assessment of angiogenesis is subject to interpreter bias.

Kornowski and colleagues from the Epstein laboratory at Washington Medical Center used the porcine ameroid model of regional stress-induced myocardial ischemia and normal pigs to evaluate transendocardial delivery of AdVEGF-121 [6]. Using an electromagnetic-based catheter guidance system, recombinant adenovirus encoding either lacZ or AdVEGF-121, injected at two to six sites at a dose of \(1 \times 10^{10}\) virus particles (vp) per site, were delivered through a retractable needle without thoracotomy. Discrete injection sites exhibited VEGF-121 expression comparable to that obtained by transepicardial AdVEGF-121 gene transfer at thoracotomy. Transgene expression was mainly adjacent to the site of injection, and 5–10% of injections showed no detectable gene transfer, perhaps due to systemic delivery of the vector. The study showed that the approach could be used to obtain gene transfer. However, there were no data regarding histological changes in the heart, regional myocardial perfusion and function, or angiogenesis.

2.2. Clinical trials

A clinical trial of patients undergoing thoracotomy and direct intramyocardial needle injection of an angiogenic gene were reported by the Rosengart and Crystal group [7]. Direct myocardial injections of AdVEGF-121 were administered to patients that were undergoing coronary artery bypass graft (CABG) surgery (Group A, \(n=21\)) and to patients as sole therapy via a minimally invasive thoracotomy (Group B, \(n=6\)). Patients with demonstrable reversible myocardial ischemia were enrolled. In patients that were to receive gene transfer in conjunction with CABG surgery, entry criteria included a left ventricular ejection fraction \(\geq 25\%\) and at least one bypassable vessel. Group B patients (gene transfer as sole therapy) were required to have a left ventricular ejection fraction \(\geq 30\%\) and be unsuitable for traditional revascularization.

A fixed volume (100 \(\mu l\)) containing AdVEGF-121 was injected in equal amounts in ten sites in a region of reversible ischemia. Five dose groups containing three patients each were evaluated for Group A (\(4 \times 10^5, 4 \times 10^8, 4 \times 10^9\), and \(4 \times 10^{10}\) vp). Patients in Group B received a dose of \(4 \times 10^9\) vp. Because this was a Phase 1 clinical trial, the principal end-points were related to safety. However, also reported were symptoms of angina, exercise treadmill testing, and regional perfusion and function assessed by nuclear imaging.

Three of fifteen patients in Group A died (20%). Group B patients were all alive at a mean follow-up of nearly 6 months. Angina class scores improved in all patients in Group A, expected sequelae of thoracotomy and CABG surgery. All patients in Group B reported reduced angina, but a statistical analysis was not performed. Coronary angiograms, read by three blinded reviewers, exhibited trends toward improvement for both Rentrop scores and collateral scores in both groups A and B, but statistically significant improvements were not obtained and no controls were included. Radionuclide perfusion imaging (with adenosine infusion) revealed no improvement in relative blood flow in the area of vector administration 30 days after gene transfer in either group. However, trends for improvement in wall motion during stress were reported for both groups. Group A patients showed no improvement in treadmill exercise 30 days after gene transfer, while trends for improvement in exercise duration, rate pressure product and ST segment/heart rate slope were reported for Group B.

The failure of both Group A and Group B patients to show improved exercise duration despite reduction in angina deserves comment. Many would argue that while the placebo effect affects treadmill performance, it might affect symptoms even more. In addition, since examiners collecting data regarding angina frequency were not blinded, the acquisition of data itself may have been biased. Indeed, the major limitation of this study is the absence of control subjects. Conclusions cannot be drawn from the study with respect to proof of concept because of this shortcoming. This criticism applies also to the studies examining angiographic collateral flow. Those analyzing the data were susceptible to bias by knowing that all patients received gene transfer. A blinded study could potentially be achieved by catheter-based methods of gene transfer not requiring thoracotomy, thereby also circumventing the confounding influence of concomitant thoracotomy and CABG surgery. A trial of this nature is currently underway.

3. Intramuscular injection of plasmid DNA

3.1. Animal studies

We will now examine the literature regarding the use of plasmid vectors encoding angiogenic genes as a means to treat heart disease. Drs. Losordo, Symes and Isner of St. Elizabeth’s Hospital in Boston have conducted the majority of these studies.

Using the porcine ameroid model of stress-induced regional myocardial ischemia they asked whether in-
tramyocardial injection of a plasmid vector encoding VEGF-165 (phVEGF-165) would increase regional myocardial blood flow [8]. Studies of ischemia were limited to microsphere assessment of regional blood flow at rest and during adenosine infusion. Gene transfer was achieved by minimally invasive thoracotomy and needle insertion into the wall of the heart in which four injections were made, delivering a total of 200 μg of plasmid in a volume of 2.0 ml (0.5 ml per injection site). Injections were performed 3–4 weeks after ameroid placement, following initial determination of basal and adenosine-stimulated regional myocardial blood flow (colored microspheres). Control animals received a plasmid vector encoding lacZ in the same quantities.

The studies demonstrated the feasibility of using thoracotomy to administer the plasmid to the heart, and also showed increases in plasma VEGF. The studies of regional myocardial blood flow were not ideal: the studies were conducted in anesthetized animals where differences in level of anesthesia could affect heart function and blood flow independently of the effect of the injected gene. In addition, the myocardial blood flow induced by a suboptimal dose of adenosine (6 mg total i.v.) was less than 50% of maximal. Even so, the authors were able to demonstrate increased left circumflex blood flow during adenosine infusion after gene transfer as compared to before gene transfer. The control group, subjected to a similar protocol, did not show such changes. When one considers that the actual degree of gene transfer with this method is substantially less than that achieved with other vectors and routes of delivery, the results are truly impressive and indicate that small amounts of transgene protein can have physiologically meaningful effects.

Returning to the effects of angiogenic gene transfer in the setting of myocardial infarction, Kloner and colleagues at Good Samaritan Hospital in Los Angeles asked whether phVEGF-165 injected into the border zone of infarcted rat heart would affect regional blood flow [9]. Rats underwent coronary occlusion of the left coronary artery followed 30 days later by an additional thoracotomy during which phVEGF-165 was injected in the peri-infarct border at one location and either saline or a control plasmid at another region of the peri-infarct border in the same heart. The blood flow was measured (microspheres) 30 days later and hearts examined.

The sites that had been injected with phVEGF-165 showed angioama formation evident on gross inspection — control plasmid and saline injected hearts did not show angioama formation. While the epicardium at the region of injection showed increased vascular structures (including angiomias), increased myocardial blood flow was not present. In addition, phVEGF-165 injection was associated with increased peri-operative death rate (death within 24 h of injection). It would be interesting to see whether the same findings would result after gene transfer using a different vector, a different isoform of VEGF, or an FGF.

While not an example of plasmid gene transfer, it is fitting to discuss a paper from Blau’s Stanford group here. They injected murine myoblasts expressing a murine VEGF analogous to human VEGF-165 into the hearts of otherwise normal but immunodeficient mice [10]. Fifteen days later five of eleven animals had died (no animals receiving similar implants of myoblasts expressing lacZ had died). Surviving VEGF-myoblast-treated animals showed intramyocardial tumors resembling hemangiomas — abnormalities not seen in the control group. The models employed by the Kloner and Blau groups may not hold much fidelity with the proposed use of VEGF in clinical settings, but their findings are consistent and bothersome.

The angiogenic potential of human hepatocyte growth factor (HGF) gene transfer was recently studied in normal and infarcted myocardium of rats by Aoki and colleagues at Osaka University Medical School [11]. HGF or control vectors were injected into rat heart at thoracotomy — the hemagglutinating virus of Japan (HJV)-liposome-plasmid vector was used. Transfection of the HGF gene resulted in increased immunoreactive HGF and PCNA-positive endothelial cells compared to control vector. HGF gene transfer, in both normal and infarcted myocardium, activated the angiogenic transcription factor, etc. Reduced HGF concentration in infarcted heart increased to normal levels 4 days after HGF gene transfer. Angiogenesis was assessed by light microscopic analysis of heart samples following perfusion–fixation. In both normal and infarcted rat hearts, HGF-treated animals exhibited greater numbers of vessels per section than animals that had received injections of control vector. Surface myocardial blood flow was assessed by laser Doppler at thoracotomy, and revealed higher values for HGF-treated normal and infarcted rats compared to controls. This was an excellent study that documented angiogenesis in a blinded and controlled manner.

Transmyocardial laser revascularization (TMR) has been recently promoted as an effective way to treat patients with angina. However, little evidence supports its utility in improving regional myocardial blood flow in randomized controlled trials. Sayeed-Shah and colleagues in Laurence Cohn’s laboratory at Harvard Medical School used TMR combined with intramyocardial injections of phVEGF-165 in the porcine ameroid model of stress-induced regional myocardial ischemia [12]. They studied six groups of animals including: ischemic controls, normal pigs, TMR only, TMR with injections of a plasmid encoding lacZ (ph.lacZ), phVEGF-165 injections only, and TMR combined with phVEGF-165 injections. Fifteen to eighteen TMR sites were created per heart and three intramyocardial injections were made within 4 mm of each TMR site in the region perfused by the left circumflex coronary artery. Each injection contained 100 μg of phVEGF-165 or ph.lacZ. Transesophageal echocardiography during rapid atrial pacing was used to assess wall motion in the ischemic region. They reported increased lacZ expression.
when plasmid injections were associated with TMR. Two weeks after treatment animals that had received combined treatment with TMR and ph.VEGF-165 injections showed improved wall motion compared with ischemic controls. However, data supporting an angiogenic effect were limited. The cardiologist analyzing the echocardiographic images was blinded, but it is not clear that images were acquired in a blinded manner. There was inadequate detail of the echocardiographic studies to evaluate the study. Sample size (n=3 in combined treatment, n=5 in ischemic controls) was inadequate to perform statistical analysis, and no conclusions can be drawn from these data.

3.2. Clinical trials

Losordo, Isner and colleagues reported on five patients with inoperable coronary artery disease and symptomatic myocardial ischemia who underwent minimally invasive thoracotomy during which 125 μg of ph.VEGF-165 was injected into the anterolateral left ventricular free wall in four 2.0-ml injections containing equal aliquots of the vector [13]. Patients had multivessel coronary artery disease, severe angina refractive to medical therapy, areas of viable but poorly perfused myocardium and left ventricular ejection fractions ≥20%.

Radionuclide imaging was conducted before, 30 and 60 days after gene transfer. Perfusion scans showed fewer abnormally perfused segments and decreased segments with fixed defects. Selective coronary angiography before and 60 days after gene transfer showed increased contrast flow (Rentrop score). All five patients reported a reduction in angina, and nitroglycerine use was reduced by day 60.

These encouraging findings are limited by the small sample size and the absence of a control group. It is argued that it is unethical to perform minimally invasive thoracotomy without providing treatment. This argument presupposes that it is appropriate to perform minimally invasive thoracotomy to treat with an unproven experimental agent in a manner that precludes the acquisition of conclusive data, a concept apparently sanctioned by regulatory agencies. A potentially suitable solution to this dilemma would be to change the patient population to include patients that undergo thoracotomy and CABG surgery, providing gene transfer in a randomized manner in a portion of the patients and not in others. Alternatively, the injections into the wall of the heart could be performed without surgery, through catheter-based methods, and a control group included, a study that was recently initiated under Losordo and Isner’s direction.

A second paper from this same group appears to be an extension of the first clinical trial with a larger sample size [14]. The study population, as before, included patients with reversible perfusion defects unsuitable for traditional revascularization. Either 125 μg (n=10) or 250 μg (n=10) of ph.VEGF-121 was injected directly into the myocardium via a minimally invasive thoracotomy, following the same injection procedure as in the previous study. There was no control group.

Patients had uneventful postoperative courses. One patient died 4 months after gene transfer. Plasma VEGF increased at 14 days to a level two-fold over pretreatment values and returned to baseline by 3 months. Patients followed for 90 days (n=16) all reported decreased angina and reduced nitroglycerine use. Seven of ten patients followed for 6 months were free of angina. Radionuclide perfusion imaging showed some improvement after treatment. The problem affecting all of the findings of this study is the absence of a control group. These data, therefore, do not provide rigorous demonstration of successful angiogenic gene therapy.

Finally, an additional study from the same group, employing similar methods, reports results in thirteen consecutive patients studied with electromechanical mapping and perfusion imaging before and after therapy [15]. The study found improvements in both parameters. Again, the studies did not include a control group and were not blinded.

4. Intrapericardial delivery of adenovirus

4.1. Animal study

In an interesting departure from intramyocardial injection of vectors, Lazarous, Unger and colleagues at the National Institutes of Health explored the effects on collateral vessel development of intrapericardial delivery of an adenovirus encoding VEGF-165 (Ad.VEGF-165) using the ameroid model of ischemia in dogs [16]. Ten days after ameroid placement, at a time when endogenous collateral formation is incomplete, Ad.VEGF165 (6×10⁹ pfu; n=9), an adenovirus encoding lacZ (6×10⁹ pfu; n=9) or saline (n=9) were injected into the pericardial space through an indwelling catheter placed at the initial thoracotomy. Twenty-eight days later maximal myocardial blood flow was measured using the microsphere technique.

Gene expression was abundant in pericardium and epicardium but not evident in the midwall or endocardium. VEGF expression was detectable in samples of fluid from the pericardial space with a peak occurring 3 days after gene transfer with subsequent decline. Plasma VEGF was not increased. Maximal myocardial blood flow was equivalent in all groups and unchanged by VEGF gene transfer. Thus, despite sustained increased amounts of VEGF produced and released into the pericardial space and gene transfer in pericardium and epicardium, increased myocardial blood flow did not occur. Of note, Ad.VEGF165 gene transfer but not Ad.lacZ gene transfer was associated with large pericardial effusions, requiring drainage and resulting in death due to pericardial tamponade in one animal. The authors suggested that large pericardial effusions might be a result of the effect of VEGF on permeability.
5. Intracoronary delivery of recombinant adenovirus

5.1. Animal studies

Hammond’s laboratory at the VA Hospital and UCSD used the porcine ameroid model of stress-induced regional myocardial ischemia and performed intracoronary injection of a recombinant adenovirus expressing a human fibroblast growth factor (Ad.FGF-5) [1]. The study compared the efficacy of intracoronary Ad.FGF5 (n=16) to intracoronary adenovirus encoding lacZ (n=7) both at 2×10^{11} vp in the treatment of already existing stress-induced regional myocardial ischemia.

Treatment was associated with mRNA and protein expression of the transferred gene in the heart. Two weeks after gene transfer, regional abnormalities in stress-induced function and perfusion were eradicated (examined by transthoracic echocardiography), effects that persisted for at least 12 weeks. Improved function and perfusion were associated with evidence of angiogenesis. This report documented, for the first time, successful treatment of abnormalities in myocardial blood flow and function following gene transfer.

The major limitation to the study was that while capillary angiogenesis was documented, evidence for increased numbers of larger caliber vessels was not reported. In fact, there were data from three animals in both groups indicating a two-fold increase in vessels >10 μm in diameter, suggesting that conduit vessels were substantially increased in animals receiving Ad.FGF-5, but the sample size was too small to perform meaningful statistical analyses (unpublished data). However, all aspects of the published study, including histological assessment of vessel number, were performed in a blinded manner.

Data reported by McKirnan et al., from the same laboratory, examined the efficacy of intracoronary delivery of an adenovirus encoding FGF-4 (Ad.FGF-4) in the setting of heart failure. It had previously been shown that the pacing-induced model of heart failure was associated with reduced myocardial blood flow per beat [17]. Therefore, a randomized, placebo-controlled and blinded study of sixteen pigs instrumented for hemodynamic monitoring and implanted with ventricular pacemakers to induce dilated heart failure was conducted [18]. Hemodynamic measures and left ventricular function (echocardiography) were measured and animals then received intracoronary delivery of 10^{12} vp of Ad.FGF-4 (n=8) or saline (n=8) in divided doses injected into the left anterior descending, left circumflex, and right coronary arteries. Pacing then was begun (HR 220 bpm) to induce heart failure, and hemodynamic measures and left ventricular function and perfusion were assessed again after 2 and 3 weeks of pacing. Animals had evidence of severe heart failure 2–3 weeks after initiation of pacing (increased left atrial pressure, dilated poorly functioning hearts). Animals that had been treated with Ad.FGF-4 showed improved regional cardiac function under basal conditions and during dobutamine infusion. FGF-4 gene transfer reduced left ventricular dimension over the course of the 3-week study. However, no differences in regional perfusion were detected. The data suggest a protective effect of FGF growth factors against ischemic injury that is consistent with a report in another model of myocardial hypoperfusion [19].

There are two limitations to these studies. First, because of limitations of the model, coronary blood flow during left ventricular pacing and regional function could not be measured simultaneously. Thus, it is possible that changes in blood flow associated with Ad.FGF-4 went undetected. Second, gene transfer was performed prior to the development of heart failure to ensure that transgene expression would be substantial when heart failure developed. It remains to be seen whether treatment of already existing heart failure can also be achieved with this method.

5.2. Clinical trials

A Phase 1/Phase 2 clinical trial of intracoronary administration of Ad.FGF-4 was initiated in May 1998 and the initial results announced March 2000. Patients with stable Class II or III angina were enrolled in a placebo-controlled (saline) double-blinded and randomized trial of the safety and efficacy of increasing doses of Ad.FGF-4 (3×10^{10}–10^{11} vp) delivered by intracoronary injection (nonsurgical). The chief end-point was treadmill exercise before and 4 and 12 weeks after treatment. Berlex Biosciences and Collateral Therapeutics, Incorporated collaborated on the filing of the commercial IND and the conduct of the trial. Berlex is the US affiliate of Schering, Berlin. Because the results of the trial have not yet been published in a peer-reviewed journal, no specific details can be provided in this review. However, preliminary results of this trial have provoked planning for more extensive clinical trials to be conducted in medical centers throughout the US and Europe.

6. Conclusions

6.1. Animal studies

It is reasonable to conclude that intracoronary and intramuscular injection of adenovirus expressing angiogenic genes can favorably affect regional myocardial function in the setting of myocardial ischemia and heart failure in animal models, as these studies were conducted in blinded and placebo-controlled investigations. It is also possible that plasmid injection of VEGF DNA into heart muscle increases myocardial blood flow. Gene transfer into the pericardial space provided increased levels of VEGF in the pericardial space, but transgene expression was appar-
ently inadequate to increase myocardial blood flow. Intramuscular injection of Ad.FGF-1 into the heart reduced the bed at risk during subsequent coronary artery occlusion, suggesting a potential protective effect of angiogenic gene transfer in subsequent acute coronary occlusion, but when ph.VEGF-165 was injected in the border region of healed myocardial infarction, blood flow was not increased and angioma formation resulted. A similar study also showed angioma formation, while plasmid-HVJ HGF injection showed no angioma formation and increased vessel counts.

On balance, these data are supportive of the concept that angiogenic gene transfer is useful in treating heart disease, but data are not uniformly positive. Instead, they indicate that the model, vector, and route of administration are critical elements of success, concepts that are predictable.

6.2. Clinical trials

A review of the current published clinical literature on angiogenic gene therapy for the treatment of myocardial ischemia does not reveal a single randomized, placebo-controlled trial. Based on current clinical literature, it is an unproven therapy. Successful animal studies combined with published reports of good outcomes in patients enrolled in uncontrolled trials has led to the expectation that angiogenic gene therapy will ultimately become a clinical reality. The next important landmark in the field will be the publication of data showing a favorable effect of angiogenic gene transfer in placebo-controlled, blinded clinical trials.

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