Gene Therapy for Restenosis
Are We Ready?

Mary Beth DeYoung, David A. Dichek

Abstract—The application of gene therapy techniques to the clinical problem of coronary restenosis has generated tremendous attention and enthusiasm. Use of gene transfer technology to prevent a common intractable illness would represent a watershed event for human gene therapy. However, the time is not yet right to initiate gene therapy trials for restenosis. The biology of restenosis is incompletely understood, catheter-based gene delivery is poorly adapted to the coronary circulation, and current gene transfer vectors are ill-suited for safe and effective gene delivery to the coronary artery wall. Basic research designed to overcome these obstacles is currently more appropriate than the initiation of clinical trials. (Circ Res. 1998;82:306-313.)

Key Words: gene therapy □ restenosis □ adenovirus

After years of unsuccessful clinical trials of traditional pharmacological agents and devices, four interventions have recently been shown to decrease restenosis. In both the STRESS (Stent Restenosis Study) and BENESTENT (Belgian Netherlands Stent Study) trials, intracoronary stent placement reduced angiographic restenosis rates to as low as 13%.1-5 Their efficacy, however, is limited by recurrence of the treated lesion in 20% to 50% of procedures. Although it is usually not immediately life-threatening, restenosis, like the initial stenosis, requires treatment to relieve symptoms (angina and exercise intolerance) and to improve the quality of life. The economic costs of restenosis are substantial. A treatment that decreased the rate of restenosis by 33% could save $2000 in health care costs per patient, adding up to more than $600 million each year.6 The development of interventions to decrease restenosis rates is a medical as well as a financial imperative.

Transcatheter-based percutaneous interventions such as balloon angioplasty and atherectomy are remarkably effective treatments for severe, symptomatic coronary artery stenosis.1-3 Their efficacy, however, is limited by recurrence of the treated lesion in 20% to 50% of procedures. Although it is usually not immediately life-threatening, restenosis, like the initial stenosis, requires treatment to relieve symptoms (angina and exercise intolerance) and to improve the quality of life. The economic costs of restenosis are substantial. A treatment that decreased the rate of restenosis by 33% could save $2000 in health care costs per patient, adding up to more than $600 million each year.7 The development of interventions to decrease restenosis rates is a medical as well as a financial imperative.

After years of unsuccessful clinical trials of traditional pharmacological agents and devices, four interventions have recently been shown to decrease restenosis. In both the STRESS (Stent Restenosis Study) and BENESTENT (Belgian Netherlands Stent Study) trials, intracoronary stent placement reduced angiographic restenosis rates to as low as 13%.8-9 Infusion of an antibody to the platelet fibrinogen receptor (glycoprotein IIb/IIIa, also known as the integrin αIIbβIIIa) also decreased clinical restenosis rates to 16%,9 and oral administration of either trapidil (a platelet-derived growth factor antagonist) or probucol (an antioxidant) has also shown efficacy in reducing restenosis rates.10,11 Although these reports are encouraging, they leave room for improved therapies that would further decrease restenosis or eliminate it entirely. Both physicians and patients look forward to the day that coronary angioplasty is a cure for symptomatic stenosis rather than merely a temporizing procedure.

Against this background, gene therapy has emerged as a novel and promising approach for preventing coronary restenosis. The attractiveness of gene therapy is based on several widely held perceptions. First, gene therapy appears capable of delivering therapeutic agents specifically to the location of the disease, at a precise site in the arterial wall. Maximal therapeutic efficacy might be achieved with minimal systemic side effects. Second, gene therapy proposes a biological solution to an essentially biological problem: regrowth of intimal mass or artery wall remodeling. Because restenosis is fundamentally the manifestation of a failed mechanical solution to a biological problem, a biological approach is intuitively attractive. Third, certain gene therapy approaches appear capable of precisely treating excessive vascular cell proliferation, potentially a key component of the pathophysiology of restenosis. Fourth, gene therapy approaches have appeared imminently applicable to large populations.

All four of these perceptions are based on solid experimental data produced either in vitro or in experimental animals.12-20 When considered together, and particularly when juxtaposed alongside the failure of traditional pharmacotherapeutics to eliminate restenosis, these perceptions have engendered great expectations concerning the current potential of gene therapy for coronary restenosis. Nevertheless, the time is not yet right for gene therapy for restenosis. The pathophysiology of restenosis is incompletely understood, the technical barriers to achieving robust intracoronary gene delivery have not been overcome, the current utility of gene transfer vectors for effective human coronary delivery is low, and the potential for harmful side effects of coronary gene delivery is high.
Selected Abbreviations and Acronyms

- ΔRb = dominant-negative (nonphosphorylatable) retinoblastoma gene
- AAV = adeno-associated virus
- HVJ = hemagglutinating virus of Japan
- NOS = NO synthase
- VEGF = vascular endothelial cell growth factor

Development of Gene Therapy for Restenosis

A complete understanding of the pathophysiology of restenosis is essential for evaluating gene therapy approaches. Unfortunately, both the biological stimuli that initiate restenosis and the molecular and cellular mechanisms by which it occurs are incompletely understood. Before the advent of gene therapy approaches, numerous clinical trials for restenosis prevention were carried out using agents that had been effective in treating other forms of cardiovascular disease. Antiplatelet agents, anticoagulants, angiotensin-converting enzyme inhibitors, calcium channel blockers, and lipid-lowering agents were all evaluated for their activities against restenosis. All of these agents failed to reduce restenosis rates. Because of these failures, investigators refocused their efforts on animal models of restenosis to elucidate the pathophysiology and to use this knowledge to develop new therapeutic approaches. Notably, these efforts centered on inhibiting smooth muscle cell proliferation, since excessive cellular proliferation was found in all animal models of balloon arterial injury. Cellular proliferation was also believed to be the primary pathological process in human coronary restenosis. Indeed, from 1991 to 1993, references to “the documented importance of smooth muscle cell proliferation in the process of restenosis” and affirmations that “smooth muscle cell proliferation seems to play a pivotal role in the restenosis process” were commonplace. Although this focus on cellular proliferation may have been inappropriate, it nevertheless guided the initial development of gene therapy approaches.

The first initiatives in gene therapy for restenosis were aimed at elimination of proliferation in the artery wall. These initiatives were inspired and facilitated by advances in biology and biotechnology including the following: the development of vector systems capable of transferring genes to the artery wall with reasonable efficiency; the development and clinical application of percutaneous catheters that might also be used to deliver genes to the artery wall; achievement of an expanded understanding of the mammalian cell cycle, including the identification of gene products that could arrest progression through the cell cycle; and, the development, largely in association with antineoplastic therapies, of therapeutic approaches that combine gene transfer with administration of a prodrug designed to kill dividing cells selectively.

Inspired by these advances, several groups developed and reported preclinical successes of gene therapy for “restenosis” in animal models. Although each of these studies has made important contributions to the field of vascular gene transfer and to the eventual development of vascular gene therapy, their contribution to the development of imminently useful treatments for human restenosis remains uncertain. When considered together, these studies raise four general questions regarding gene therapy approaches to prevent restenosis: (1) Is gene therapy a rational approach to reversing the biological processes that produce human coronary restenosis? (2) Is catheter-based gene therapy for coronary restenosis technically feasible? (3) Are current gene transfer vectors suitable for delivery into human coronary arteries? (4) Is coronary restenosis an optimal target for an early gene therapy trial?
such as stenting may offer a more logical approach to preventing restenosis of severely diseased arteries.

A fourth potential weakness, based again on biological considerations, is that several proposed gene therapies for restenosis may interfere with normal arterial function. The epicardial coronary arteries are metabolically active organs that regulate myocardial blood flow by dilating and contracting in response to physiological stimuli. Interventions that interfere with cellular proliferation and disrupt important cellular pathways could produce more harm than benefit. The use of a dominant-negative Ras mutant to suppress intimal thickening illustrates the potential risks of interfering with arterial cell metabolism to suppress restenosis. Ras is a component of intracellular signaling pathways that regulate transcription of a wide variety of cellular genes, including β-myosin heavy chain, skeletal and cardiac α-actin, and plasminogen activator inhibitor type 1. Inhibiting Ras function by gene therapy may interfere with intimal thickening, but it is also likely to have pleiotropic effects on vascular smooth muscle cell function. Use of ΔRb for restenosis gene therapy may also have unwanted effects. Although it is clear that ΔRb can inhibit cell cycle progression in vitro and prevent intimal thickening in animal models of balloon arterial injury, ΔRb may also suppress the transcription of ribosomal RNA genes. A decrease in ribosomal RNA expression might have more generally negative effects on cellular protein synthesis and normal cellular physiology. It is not clear (and therefore should be demonstrated) that arteries expressing significant amounts of ΔRb or dominant-negative Ras will function physiologically. Finally, gene therapy with NOS, which decreases intimal proliferation in vitro and prevent intimal thickening in animal models of balloon arterial injury, might interfere with intimal thickening, but it is also likely to have pleiotropic effects on vascular smooth muscle cell function.

In summary, current gene therapy approaches to restenosis may be based on inappropriate animal models and on questionable views of the importance of smooth muscle cell proliferation in human restenosis. Moreover, many of these approaches make use of cytotoxic and cytostatic genes that are not likely to prevent the vascular remodeling and contraction processes that are the primary determinants of coronary restenosis. Indeed, expression of certain genes might disrupt normal vascular function and could even be proatherogenic. It seems apparent that our understanding of the biology of coronary restenosis is too rudimentary for confident identification of specific therapeutic genes as effective mediators of gene therapy.

Is Catheter-Based Gene Therapy for Coronary Restenosis Technically Feasible?

The primary attraction of angioplasty as an alternative to coronary artery bypass surgery is that it is performed as a minimally invasive procedure. To be compatible with coronary angioplasty, gene therapy for restenosis must also be administered conveniently and effectively at the tip of a catheter. Catheters have been a prominent part of vascular gene therapy research, beginning with the first study demonstrating in vivo arterial gene delivery. The prominence of familiar, clinically applicable catheter devices in animal vascular gene therapy studies has helped foster the general impression that clinical vascular gene therapy trials in humans are imminent. Unfortunately, this impression is likely incorrect. Effective percutaneous gene delivery to coronary arteries remains a distant goal, in part because a suitable catheter has not yet been developed.

The double-balloon catheter was the first device used to infuse recombinant genes into the arterial wall in animal model systems. On the basis of histochemical staining in these initial experiments and the ability of this system to produce biological effects in subsequent experiments, the double-balloon catheter appeared to be a potentially useful tool for coronary gene delivery. However, in these studies the catheter was inserted into a branch of the pig iliofemoral artery under direct vision after surgical isolation of the artery and ligation of side branches. Human coronary arteries have far more branches than the pig iliofemoral artery, and none of these branches can (or should) be ligated at the time of percutaneous delivery.

Deployment of a double-balloon catheter in the coronary circulation would likely result in inefficient local gene delivery and the systemic release of vector-containing solution via the numerous intramyocardial side branches. In addition, use of the double-balloon catheter would completely occlude the coronary lumen during gene delivery. It is likely that this prolonged occlusion (typically 30 minutes) would produce intolerable myocardial ischemia. Notably, the vast majority of vascular gene transfer and gene therapy studies in animals have been performed in segments of iliofemoral and carotid arteries, usually with surgical rather than percutaneous approaches. These arterial segments have few or no side branches and serve territories that are well supplied with collateral vessels. These experimental approaches have been highly informative, but they do not confront the enormous technical challenge of achieving efficient percutaneous delivery into a highly branched coronary artery serving an ischemic territory that has inadequate collateral circulation.

Other catheters used for gene delivery include perforated-balloon catheters, modified perfusion balloon catheters, and gel-coated catheters. Perforated-balloon catheters, in which vector solutions are injected into the artery wall through small holes in a balloon, offer the potential to deliver genetic material over periods as short as 5 to 10 seconds, minimizing the duration of occlusion. However, the pattern of gene delivery with these catheters is uneven, and the high-pressure jets of vector can damage the artery wall. Microporous balloon catheters, in which infusion ports are more numerous and more evenly distributed, may overcome these problems by permitting infusion of vector-containing solutions at a lower, less destructive pressure. The gel-coated catheter, in which genetic material is incorporated into a gel that coats an angioplasty balloon tip, permits gene delivery at the same time as angioplasty; however, this system appears to permit only a very low efficiency of gene transfer. Certain other infusion catheters...
contain a central lumen, permitting downstream perfusion during gene delivery.\textsuperscript{13,67} Despite these advances in catheter design, no catheter–based system yet described appears likely to achieve reliable and efficient gene delivery to the coronary artery wall.

Are Current Gene Transfer Vectors Suitable for Delivery Into Human Coronary Arteries? A suitable gene transfer vector for restenosis gene therapy would have the following characteristics: (1) high efficiency as measured by the ratio of cells expressing a recombinant gene to total cells exposed to the vector, (2) ability to achieve recombinant gene expression from nondividing cells (the baseline mitotic rate in the coronary artery wall is <1\% even in advanced lesions\textsuperscript{41,75}), (3) minimal vascular toxicity from direct exposure to the vector or from an immune response against vector-transduced cells, (4) absence of baseline immunity to the vector in the majority of the population, and (5) sufficient duration of recombinant gene expression to prevent restenosis. This last characteristic is vague by necessity, as it is not clear over what time period gene therapy must be administered to prevent coronary restenosis. Although tremendous progress has been made in vector development for vascular gene therapy, no currently available vector satisfies all of these characteristics.

Plasmid DNA and retroviral vectors were the first agents used for vascular gene delivery.\textsuperscript{12} Because of their extremely low efficiencies (well below 1\% in vivo)\textsuperscript{44–46,72} and, in the case of retroviral vectors, their inability to transfer genes into nondividing cells, these agents appear to have been nearly abandoned as experimental vascular gene delivery vectors. Notably, an exception to this abandonment is a human gene therapy trial in which a plasmid encoding VEGF is used to treat peripheral arterial disease.\textsuperscript{73} It will be of interest to follow whether the successes reported by this group\textsuperscript{74–76} lead to an expanded use of plasmid DNA for arterial gene delivery. Despite intimations that VEGF might prevent intimal thickening,\textsuperscript{77} VEGF gene delivery does not currently appear destined for application to human coronary restenosis. Two independent studies suggest that VEGF delivery may actually worsen arterial intimal hyperplasia.\textsuperscript{78–79}

The initial descriptions of high-efficiency in vivo vascular gene delivery by adenoviral vectors\textsuperscript{15,17,61} provided a huge impetus in the development of gene therapy for restenosis. The “advent of adenovirus” was heralded as a major turning point in cardiovascular gene therapy,\textsuperscript{80} and important biological and preclinical vascular gene transfer studies performed with adenoviral vectors would not have been possible using other less efficient vector systems.\textsuperscript{10,19,39,65,66,81–83} Adenovirus continues to be an extremely useful tool for vascular gene delivery in animal models. However, it has severe limitations as an agent for vascular gene therapy in humans.

As agents for vascular gene delivery, adenoviral vectors are limited by the following: (1) the high prevalence of preexisting immunity to adenovirus, (2) the profound destructive immune response generated to adenovirus–transduced cells, and (3) direct tissue toxicity. These limitations have become evident as a result of extensive work performed by several groups primarily involving liver and lung gene transfer systems\textsuperscript{84–87} but also involving vascular gene delivery.\textsuperscript{88–90}

The problem of preexisting immunity (limitation 1) was inapparent for several years primarily because adenoviral gene delivery studies were performed in laboratory animals without prior exposure to human adenovirus. Because they are uniformly seronegative for human adenovirus exposure, laboratory animals are highly susceptible to gene transfer on a first exposure to adenoviral vectors. However, once immunized with adenoviral vectors, they essentially cannot undergo successful vascular gene delivery.\textsuperscript{81} This barrier can be partially overcome by suppression of the cellular immune system with agents such as cyclosporine A. Unlike laboratory animals, adult humans have a high prevalence (\textasciitilde 60\%) of seropositivity to adenovirus\textsuperscript{91} and an even higher prevalence of memory T-cell response to adenovirus (95\%).\textsuperscript{92} In the absence of concurrent immunosuppression, vascular gene delivery with adenoviral vectors may not be possible in the vast majority of coronary artery disease patients. In this case, the risks of immunosuppression (as well as other risks of gene delivery mentioned above and below) must be balanced against the anticipated risk of restenosis.

In the absence of continuous immunosuppression, even when efficient vascular gene delivery can be obtained with adenoviral vectors, recombinant gene expression will likely be short-lived. Transduced cells, which express low amounts of adenoviral proteins encoded by the vector backbone, are eliminated by the host immune response. This focused immune response (limitation 2) results not only in the death of transduced cells and cessation of recombinant gene expression but also results in the presence of inflammatory cell infiltration and vascular cell activation in the artery wall. Inflammation and vascular cell activation, as measured by increases in expression of intercellular adhesion molecule 1 and vascular cell adhesion molecule 1, are thought to play an integral role in the pathogenesis of atherosclerosis.\textsuperscript{93–95} The very condition that gene therapy is intended to treat. Of greater concern, increased infiltration of inflammatory cells in atherosclerotic arteries has been associated with plaque rupture, a potentially fatal complication of coronary atherosclerosis.\textsuperscript{43} Indeed, inflammation appears to play a major role in the pathogenesis of myocardial infarction and ischemic stroke.\textsuperscript{96–98} From this perspective, adenoviral gene delivery would have to offer substantial benefits to justify the potentially increased risk for worsened atherosclerosis and plaque rupture.

Direct tissue toxicity is the third serious limitation of adenoviral vectors for vascular gene delivery. When infused at high concentrations, adenoviral vector solutions cause smooth muscle cell death and endothelial denudation.\textsuperscript{80} A recent report of abnormal vascular reactivity after adenoviral gene delivery suggests that sublethal forms of vascular toxicity may also exist.\textsuperscript{89} An encouraging aspect of this particular limitation is that the most severe direct toxicity can be avoided by lowering the administered dose.\textsuperscript{97}

The above limitations of adenoviral vectors are substantial and would appear to exclude use of these vectors for human arterial gene transfer protocols. There have, however, been encouraging reports of second- and third-generation adenoviral vectors in which expression of viral genes is suppressed or even eliminated entirely\textsuperscript{97–100} and of innovative methods of subverting the host immune response to the vectors.\textsuperscript{101,102} The
most exciting of these reports describe “gutted” adenoviral vectors that are deleted of all viral genes and immunosuppressive therapies that appear to eliminate the immune response to adenoviral antigens. These approaches could potentially remove significant obstacles to the application of adenoviral vectors to human gene therapy. However, before these approaches can be tried in humans, they must first be proven in animal vascular gene delivery systems. Major issues that remain to be addressed include whether delivery of the “gutted” vectors can be accomplished in the setting of preexisting immunity and whether patients can be rendered tolerant of adenoviral vectors without increasing their susceptibility to overwhelming adenoviral infection.

Two other vector systems (AAV and the HVJ/liposome DNA system) have been used for arterial gene transfer and may eventually be useful for restenosis gene therapy. AAV vectors can produce recombinant gene expression in skeletal muscle for prolonged periods of time, without detectable inflammation. Three recent reports describe the use of AAV vectors for arterial gene delivery. Two reports were highly positive, whereas the other was less so. More extensive animal studies are required to determine the potential of AAV for arterial gene therapy. The HVJ/liposome system appears to mediate efficient arterial gene delivery, but it has been used in only a handful of laboratories. More widespread experience with this vector system would establish greater confidence that its strengths and shortcomings were fully understood. Certainly, our understanding of adenoviral gene delivery has benefited from the broad range of skills, expertise, and perspectives that has been applied to its use.

In summary, no current vector system appears ready to be applied safely and with confidence to the prevention of coronary restenosis. New and improved vectors are being described regularly; one of these vectors may provide a means for safe and effective arterial gene delivery in humans. Experiences with vectors used for arterial gene delivery to date have identified efficiency, toxicity, and interactions with the immune system as critical points to consider in future vector development.

Is Coronary Restenosis an Optimal Target for an Early Gene Therapy Trial?

In planning human trials of new biomedical technologies, such as gene therapy, several important points must be considered. How serious is the disease? Are there alternative therapies, and are these therapies sufficiently unlikely to succeed that a truly unproved (and potentially injurious) intervention is merited? Among the population of patients for whom an experimental therapy is considered, how likely is an individual patient to develop the disease? As mentioned above, restenosis is inconvenient and expensive, but it is typically not life-threatening. In this respect, it differs from three early targets of gene therapy approaches—adenosine deaminase deficiency, familial hypercholesterolemia, and metastatic brain cancer, all fatal diseases with inadequate therapeutic options. An angioplasty for symptomatic stenosis provides a definitive cure in 50% to 70% of the cases, and subsequent angioplasties of the same artery are equally likely to succeed. A strategy of repeat angioplasty yields an overall clinical success rate of 95%. Thus, most candidates for restenosis gene therapy will, in fact, never develop the disease. Under such conditions, gene therapy would need to be of proven efficacy and unquestionable safety. Clearly, this is not yet the case. In fact, the potential consequences of inappropriately applied coronary artery gene therapy could be extreme, including plaque rupture and death.

Several other questions are equally important in assessing the current suitability of gene therapy for coronary restenosis: Is the disease pathogenesis sufficiently understood that an experimental therapy is not simply a shot in the dark? Can a therapeutic trial be designed so that it is informative even if a negative result is obtained? How large and costly a trial is required to achieve a definitive result? Consideration of these issues does not engender significant optimism. Because the biological basis of restenosis is incompletely understood, selection of a gene to deliver involves a substantial component of chance. This element of randomness might be justified if a trial could be set up such that a negative result would be informative and definitive. Unfortunately, because there are currently no means of monitoring the success, efficacy, or local tissue toxicity of human coronary gene delivery, a negative result of a restenosis gene therapy trial will almost certainly be uninformative. Failure of such a trial could be due to inefficient gene delivery, inadequate gene expression, too short a duration of expression, or confounding local toxicity. Because of the inaccessibility of the coronary circulation, no data will be available to monitor any of these variables. Gene therapy for restenosis would essentially be carried out in a black box. Whereas this situation may be necessary under certain clinical circumstances, revolutionary therapeutic interventions such as gene delivery are optimally tested in settings in which negative results can be instructive, ie, settings in which the reason(s) for failure may be ascertained. A final point to consider is the magnitude and cost of a definitive study. As discussed extensively elsewhere, death, nonfatal myocardial infarction, and need for repeat revascularization occur at a fairly low frequency (20% in 6 months) in the population most likely to be included in a clinical trial of a new therapy for restenosis. To detect with confidence a modest, yet important, decrease in these “hard” end points (25% to 33%), a trial would have to enroll 1000 to 2000 patients. Identification of a funding agency willing to support the high cost of such a trial, given the uncertainties surrounding the promise of restenosis gene therapy, will be a significant challenge.

Building for the Future

Although our assessment of the current status of gene therapy for restenosis is largely pessimistic, we believe that certain research directions may eventually lead to a clinical trial. The top priority is to acquire a complete understanding of the pathogenesis of restenosis. Only by identifying the critical rate-limiting processes that cause restenosis and by clarifying the molecular components of these processes will it be possible to select appropriate candidate therapeutic genes.

Data obtained in the EPIC (Evaluation of Ib/IIa Platelet Receptor Antagonist 7E3 in Preventing Ischemic Complications) trial, in which antibodies to the platelet fibrinogen receptor glycoprotein Ib/IIa reduced clinical restenosis, suggest that it may be worthwhile to reconsider the hypothesis.
that thrombosis plays a major role in restenosis. Development and application of improved animal models of restenosis are the most expedient means to pursue this and other hypotheses. The most commonly used models of single-balloon injury to normal rat and rabbit arteries have simply not been useful in predicting the success of pharmacological interventions. Progress is also required in device and vector development. Catheters must be developed that can deliver genes efficiently without causing ischemia or vascular trauma. Alternatively, stents or polymers might be developed that are capable of delivering genes after deployment in the artery wall. In view of the potentially dangerous complications of coronary artery gene delivery, including plaque rupture and vessel occlusion, gene therapy for arterial disease is best initiated in the peripheral circulation. The recent initiation of a human vascular gene therapy trial in the peripheral arteries reflects, in this respect, appropriate exercise of caution in this uncertain therapeutic arena. Finally, the development of noninvasive means of monitoring recombinant gene expression after in vivo gene delivery would enable investigators to learn maximally from negative as well as positive therapeutic trials.

If significant progress is made along the lines described, it may some day be worthwhile to pursue gene therapy for restenosis. At present, however, gene therapy trials for restenosis are ill-advised. If initiated, such trials are far more likely to set back the gene therapy field by producing negative, uninformative, or even catastrophic results than they are to lead to important clinical progress. As always, stepwise rational progress is preferable to headlong forays into the unknown that risk lives and resources with little promise of substantial returns.

References


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