Lessons From Human Arteries
How to Design a Gene Therapy Strategy for Treatment of Cardiovascular Disease
Ryuichi Morishita

Gene therapy is emerging as a potential strategy for the treatment of cardiovascular disease, such as restenosis after angioplasty, vascular bypass graft occlusion, and transplant coronary vasculopathy, for which no known effective therapy exists. The first federally approved human gene therapy protocol started on September 14, 1990, in adenosine deaminase-deficient patients. Eight years since the commencement of the first trial, >250 clinical studies of gene therapy are under investigation. In the cardiovascular field, >5 protocols have been approved. More recently, Isner and colleagues have demonstrated the utility of gene therapy using an angiogenic growth factor (vascular endothelial growth factor [VEGF]) for the treatment of critical limb ischemia in human patients. A reendothelialization strategy using the VEGF gene has also been tested in a clinical trial for the treatment of restenosis after angioplasty. The objectives were generally to evaluate (1) the in vivo efficacy of the gene transfer method, (2) the safety of the gene transfer method, and (3) the possible therapeutic efficacy. Although there are still many unresolved issues, human gene therapy for cardiovascular disease is now becoming a reality. Nevertheless, gene therapy still requires efficient in vivo gene transfer technology to achieve the final goal. During the past decade, many gene transfer methods, including viral transfer techniques such as the adenoviral method, have been developed, and some are being applied clinically in human gene therapy studies.

In addition to these issues, it is time to take a hard look at practical issues that will determine the real clinical potential. These include (1) further innovations in gene transfer methods, (2) well-defined disease targets, (3) cell-specific targeting strategies, and (4) effective and safe delivery systems. However, it is difficult to address these issues in regard to human blood vessels despite its necessity. Thus, investigators have used animal models to test their hypotheses in relation to human clinical therapy. Although there are numerous reports of successful treatment of cardiovascular disease using gene therapy strategies in animal models, possible differences between animal experiments and real therapy in human cardiovascular vessels still exist as follows: (1) Can significant gene transfer be accomplished with human blood vessels? (2) Is there a difference between normal and atherosclerotic vessels in susceptibility to gene transfer? (3) What cell types can be readily infected in normal versus atherosclerotic vessels? (4) Do anatomic barriers (eg, endothelial cells and extracellular matrix) influence the transfection efficiency in atherosclerotic plaques?

Utility of the Organ Culture System of Human Blood Vessels in the Study of Gene Therapy
The study of Rekhter et al in this issue of Circulation Research investigates these issues using organ culture of human blood vessels. They used (1) human blood vessels from normal coronary arteries of donor heart in order to study inhibition of the formation of transplant atherosclerosis or transplant rejection, (2) normal saphenous veins and internal mammary arteries to study inhibition of coronary bypass graft intimal hyperplasia, (3) advanced atherosclerotic plaques to study stabilization and promotion of the regression of occlusive inoperable atherosclerosis, and (4) advanced, mechanically injured atherosclerotic plaques to study the prevention of restenosis after angioplasty. In particular, their studies emphasize the transfectability of nonendothelial intimal cells (smooth muscle cells and macrophages) as the most probable direct participants in pathological intimal growth and, therefore, as attractive targets for gene therapy.

Rekhter et al studied the expression pattern of adenoviral gene transfer using their own system. Somatic gene therapy consists of the introduction of normal genes into the somatic cells of patients to correct an inherited or acquired disorder through the synthesis of specific gene products in vivo. For this purpose, many in vivo gene transfer methods have been developed. In vivo gene transfer techniques for cardiovascular applications include the following: (1) viral gene transfer: retrovirus, adenovirus, or hemagglutinating virus of Japan (HVJ [Sendai virus]), (2) liposomal gene transfer: cationic liposomes, and (3) in vivo reimplantation of cells modified in vitro. These in vivo gene transfer techniques have different advantages and disadvantages. Adenovirus-mediated transfer is a promising gene transfer method for the treatment of cardiovascular disease, because the adenoviral method is very effective for transfection into nonreplicating cells, including vascular smooth muscle cells and endothelial cells in vivo as well as in vitro. In contrast, the expression is temporary (weeks to months), suggesting that this transfer method may be a useful system for the treatment of acute diseases, such as restenosis and graft failure, in which only temporary expression of the transgene is necessary. However, in vivo animal

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experiments have demonstrated that endothelial cells work as an anatomic barrier, and thereby, the transfectability into medial smooth muscle cells with adenovirus-mediated gene transfer is limited. The studies by Rekhter et al confirmed these in vivo observations from animal experiments in human normal coronary artery organ culture. They found that the recombinant gene driven by adenovirus was expressed preferentially in endothelial cells (~100%), but not intimal smooth muscle cells (~1.3% to 3.8%) in normal human vessels. However, cells adjacent to the loci of plaque rupture and cells associated with organized thrombi present in advanced and complicated plaques were more prone to gene transfer. This information is particularly important in achieving high transfection efficiency sufficient for human gene therapy, since the targets for gene therapy are not normal arteries. In addition, their data suggest a broad scope of cellular targets, including endothelial cells, smooth muscle cells, and macrophages for gene therapy in human atherosclerosis. In contrast, the depletion of macrophages before adenovirus-mediated gene transfer has been reported to increase the transduction efficiency and reduce the rate of immunological elimination of the adenovirally transduced cells, thereby increasing the persistence of transgene expression in immunocompetent animals. Thus, the suitability of macrophages as the targeted cells for adenovirus-mediated gene transfer should be further clarified.

On the other hand, the usefulness of an organ culture system of human blood vessels is noteworthy in the study of human gene therapy for bypass graft failure. Accelerated vein graft atherosclerosis remains a major limiting factor in the successful treatment of occlusive arterial disease, and attempts to date at improving long-term autologous vein graft patency with various pharmacotherapeutic agents have failed. Previously, researchers have speculated that genetic engineering may be able to improve the long-term function of vascular vein grafts that are prone to atherosclerosis and occlusion. Mann and colleagues have reported that administration of antisense proliferating cell nuclear antigen and cdc 2 kinase oligodeoxynucleotides into a vein graft model successfully inhibits neointimal formation, which is accompanied by increased resistance to diet-induced atherogenesis. In 1996, clinical application of a “decoy” against an essential transcription factor for the activation of cell cycle regulatory genes, E2F, was approved by the FDA to treat neointimal hyperplasia in vein bypass grafts, which results in failure in up to 50% of grafts within a period of 10 years. We and others have also reported a gene therapy strategy using the p21 (sd1-1) gene or an endothelial constitutive nitric oxide synthase gene to prevent neointimal formation in a rabbit vein graft model. Rekhter et al have reported a new tool for the development of a gene therapy strategy against bypass graft failure. Alternatively, the organ culture approach is attractive for the analysis of gene transfer into human atherosclerotic plaques, since advanced features of human lesions such as plaque rupture, erosion, and hemorrhage are extremely difficult to reproduce in animal models.

Anatomic Barriers for Gene Therapy in Blood Vessels

Apparently, 2 anatomic barriers (endothelial cells and extracellular matrix) exist to prevent high transfection efficiency into cellular targets in atherosclerotic vessels, as Rekhter et al have provided new insights to overcome the low transfection efficiency due to the extracellular matrix. Of importance, collagenase and elastase treatment increased the percentage of transgene expression 7-fold. These treatments may be feasible for gene therapy against bypass graft failure. In contrast, how to penetrate endothelial cells, another anatomic barrier, is still unresolved, although Rekhter et al successfully demonstrated the ability to target intimal cells without intenational endothelial denudation. It may be achieved by increasing endothelial permeability and/or increasing pressure during incubation.

More important, the publication by Channon et al, also appearing in this issue of Circulation Research, documents endothelial activation by adenovirus-mediated gene transfer in a rabbit model. Their studies showed significant time- and titer-dependent impairment of endothelium-dependent relaxation, with no effect on contraction or nitroprusside-induced relaxation. Previously, Lafont et al also reported vasomotor dysfunction early after exposure of normal rabbit arteries to an adenovirus vector. Since impairment of endothelium-dependent relaxation is well known to cause cardiovascular events, the report by Channon et al should be considered in designing gene therapy protocols. In addition, their report has also provided new insight into the immunogenicity of adenovirus-mediated gene transfer, a well-known side effect, as Channon et al have documented that the host response, rather than direct viral toxic effects, is largely responsible for functional endothelial injury. This issue is quite important, since other researchers have also reported that adenovirus-mediated gene transfer to the endothelium was much more effective in atherosclerotic than in normal vessels. Thus, further research should stimulate additional investigation into the understanding the biological properties and changing the characteristics of endothelial cells. We have used the viral-liposomal gene transfer (HVJ-liposome) method to achieve a high transfection efficiency into blood vessels across the endothelium. Previous reports have documented the transfectability of the HVJ-liposome method into both the medial and adventitial layers in vein grafts as well as an organ culture system. In addition, Yonemitsu et al documented that HVJ-liposome method could achieve highly efficient gene transfection into the medial smooth muscle cells of intact arteries at 150 and 760 mm Hg of pressure across the endothelial cells (mean, 85.3% and 93.5% of total smooth muscle cells, respectively) without any inflammatory reaction. In addition, the liposomal method, which is safe and easy to handle, seems to easily achieve penetration of the endothelial barrier, and some reports have documented that plaque lesions are favorably transfected by the liposomal method because of the affinity of liposomes for cholesterol. Comparison of the transfection efficiency using other methods in human normal versus atherosclerotic blood vessels should also be performed.
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The reports by Rekhter et al. and Channon et al. published in this issue of *Circulation Research* should stimulate additional investigations into gene therapy strategies, including (1) how to overcome the presence of permeability barriers that limit the transgenes from reaching their cellular targets, (2) how to open the plaque naturally (eg, by erosions or rupture) or artificially (by enzymatic treatment), and (3) how to maintain the endothelial cell function as a natural biological gate-keeper. Because characteristics of the efficiency and patterns of transgene expression in human vessels can provide new information in the design of gene therapy, further efforts to investigate the biology and pathophysiology of arteriosclerosis, atherosclerosis, restenosis, and graft failure should be stimulated.

References


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