Gene Therapy and Endovascular Treatment of Intracranial Aneurysms

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Background—Endovascular treatment of intracranial aneurysms is safe and effective but too often is followed by recurrences. Gene therapy may improve healing after embolization, and endovascular approaches may offer future in situ delivery systems designed to prevent aneurysm rupture.

Summary of Review—Advances in coil technology have focused on coating strategies designed to modify the biological reaction to the embolic agent. Gene therapy in cardiovascular applications is limited by low efficiency and transient gene expression. Current advances include the potential use of circulating progenitor cells for ex vivo genetic manipulations followed by in vivo delivery. Direct gene transfer may also be enhanced in situ by coils carrying antibody-tethered adenovirus or through the use of cell-specific or radiation-inducible promoters. Candidate genes that may be of value in promoting healing after endovascular treatment include growth factors and metalloproteinase inhibitors. A better understanding of the biology of aneurysm is necessary to conceive strategies designed to control the development of these lesions before their rupture.

Conclusions—Many technical difficulties remain to be solved, but the combination of gene therapy and endovascular techniques offers multiple therapeutic possibilities in the future control of intracranial aneurysms. (Stroke. 2004;35:786-793.)

Key Words: aneurysm, intracranial ■ endovascular therapy ■ gene therapy

The current alternative to surgical clipping of intracranial aneurysms is endovascular occlusion with platinum coils. Endovascular treatment is safe and effective, and it can improve the outcome of patients with ruptured aneurysms compared with surgical clipping.1-3 Coil embolization, however, is sometimes incomplete and subject to recanalization and recurrence in 20% of patients.4

In North America, 1% to 8% of the adult population bears asymptomatic intracranial aneurysms, but the incidence of aneurysmal rupture is approximately 5 to 10/10,000 per year.5-7 There is no reliable predictor of aneurysmal rupture.

Gene therapy is a technique in which a functioning gene is inserted into a cell to correct a genetic error or to introduce a new function to the cell. Lifelong replacement is required to preclude the pathological consequences of a defective gene. In cystic fibrosis or muscular dystrophy, for example, vectors should provide robust and prolonged gene expression in a large proportion of target cells or tissues. In cerebrovascular diseases, gene therapy has been considered mainly for ischemic disorders.7 Genetic approaches may provide future tools to identify patients at risk of developing an aneurysm or a means to control the development and rupture of intracranial aneurysms.

Endovascular interventions address structural consequences of the pathology rather than its biological basis. Cardiovascular gene transfer techniques usually target the local expression of a particular gene that will respond to a given punctual shortcoming, such as in restenosis.8 Transient gene expression may be sufficient to modulate a physiological response. Thus, promoting neointima formation and preventing recanalization at the neck of aneurysms after endovascular treatment are more realistic goals at present, and these applications will be the main focus of this article.

Potential Role of Gene Therapy in Controlling the Development of Intracranial Aneurysms

Genetic disorders may play a role in the development of intracranial aneurysms in some patients. Hereditary diseases associated with intracranial aneurysms include Marfan and Ehlers-Danlos (type IV) syndromes, neurofibromatosis (type I), and autosomal dominant polycystic kidney disease.9,10 Furthermore, a familial incidence not associated with connective tissue disorders is also recognized.9 A role for endoglin polymorphism is controversial.11-13 A molecular marker related to aneurysm formation would be helpful to screen a high-risk population. Another potential goal of gene therapy may be stabilization of the aneurysmal wall to prevent future ruptures, with overexpression of matrix metalloproteinase (MMP) inhibitors, for example.14-17 Unfortunately, molecu-
lar mechanisms involved in aneurysm formation, growth, and rupture remain poorly defined. Studies on tissue samples from human aneurysms inevitably deal with late stages of disease. The pertinence of many animal models, including knockout studies, remains to be clarified. The most frequently mentioned concepts regarding the pathophysiology of aneurysm evolution toward rupture involve collagen, elastin, matrix proteases, fibrinolysins, and corresponding inhibitors. Unless one identifies a common but specific defective gene that can be palliated, replacement therapy is unlikely to apply to the majority of patients at risk of developing aneurysms. These potential applications require a deeper understanding of the biological basis of aneurysm development and evolution than is currently available.

**Recurrences After Endovascular Treatment and Recent Developments**

There has been little progress in the understanding of the biological response to embolization and to healing phenomena or their deficiencies after endovascular treatment. Efforts in this field have focused on the development of new devices or on modification of coils in an attempt to change the biological reaction after embolization. Coils have been coated with extracellular matrix, nonbiodegradable or biodegradable polymers, or fibroblast-secreting growth factors or have been modified with ion implantation. Although surface modifications of coils have shown promise in some models, they have provided few insights into mechanisms responsible for recurrences. Tamatani and collaborators were the first to report the interaction of cultured endothelial cells with coated embolic agents. The following year, Kallmes et al proposed a novel treatment in which coils were used as cell delivery vehicles. Recently, alginate microspheres were proposed to deliver cells and growth factors. Coils coated with collagen/recombinant human vascular endothelial growth factor (VEGF) were also explored to enhance healing after coil embolization. The shift of interest from pure mechanical aspects of endovascular devices to a biologically integrated approach in which the embolic agent is a tool to deliver active molecules or living cells is recent but opens infinite future therapeutic possibilities. Hypothetical mechanisms involved in healing or recurrence after endovascular treatment of aneurysms are illustrated in Figure 1. Therapeutic strategies could target the promotion of neointima formation or inhibition of the recanalization process.

**In Situ Gene Therapy of Aneurysms**

The most important steps involved in gene transfer into cells are summarized in Figure 2. In situ gene therapy of aneurysms involves selection of a candidate gene, identification of target cells, and design of a means to transfer efficiently the desired gene into these cells. Vectors may be needed to transfer genes into cells, and promoter systems are required to regulate gene expression according to the therapeutic objectives. Gene insertion into somatic cells has been explored extensively for 10 years, and, more recently, stem and progenitor cells are becoming prominent vehicles to express therapeutic genes. There are, however, many technical and conceptual obstacles to be overcome before human gene therapy becomes a routine procedure.

**Vector Delivery**

The efficiency of gene therapy depends on a significant level of gene transfer and protein expression with therapeutic but not toxic effects. The relevant approach for gene transfer is sometimes obvious. For example, if a tissue-specific response is difficult to obtain with systemic administration, it becomes possible with some direct or in situ approaches, such as a bronchoscopic or intramyocardial administration. One specific challenge of vascular gene therapy is the difficulty involved in efficiently transferring the desired gene at the target site. Two methods have been proposed for vascular gene therapy: ex vivo or cell-based transfer and in vivo or direct gene transfer. The first approach uses an intermediate cellular stage and involves multiple steps (Figure 3A): (1) collecting vascular cells for in vitro expansion (eg, from the vessel wall); (2) ex vivo transduction of the cells with the use of viral or nonviral vectors; and (3) reintroduction of cells expressing the transgene into the vasculature. Ex vivo gene transfer has several advantages over in situ gene transfer. Because this technique is performed in an in vitro environment, gene transfer efficiency can be optimized; target cells can be purified, identified, and expanded; gene expression can be measured; and phenotypic changes of the target cell can be determined before reimplantation. The optimal source of vascular cells to be harvested and cultured has not yet to be determined, but many alternatives can be explored (see below). Means of reintroducing cells at the aneurysmal site are still limited, however. Reimplantation in vivo has been performed initially by coating synthetic grafts or stents with vascular cells. In experimental models, vascular smooth muscle cells (VSMCs) have been cultured onto sponges and reintroduced into aneurysms by percutaneous techniques. Others have proposed alginate as an embolic agent that could deliver cells by transcatheter techniques.

The second approach is the direct in vivo or in situ transfer of genetic material into the vessel wall (Figure 3B). In vivo gene transfer is of interest for cardiovascular diseases because of its relative simplicity. No cell harvesting or culture expansion is required. Endovascular devices initially designed for in vivo gene therapy included double balloon catheters, as well as infiltrating and hydrogel-coated balloons. In vivo gene therapy was successfully used to inhibit arterial restenosis or to promote formation of new collateral vessels in ischemic myocardium in animal models. Direct vascular gene transfer is currently limited by its low efficiency, perhaps related to concentrations and binding time of vectors. It is not possible to stop the circulation for a long time during local vascular administration, and collateral vessels dilute the vector away from the target site. Furthermore, methods to quantify vector concentration at a vascular target are at best suboptimal. Better gene delivery systems are thus needed to improve efficacy.

In situ gene therapy of aneurysms presents an added difficulty: at the time of endovascular treatment, the neck of
the aneurysm that needs to be occluded is a hole through which blood flows. The neointimal cells that could be targets for gene therapy have not yet reached the area. Only parietal cells such as endothelial cells, at the level of the aneurysm or parent vessel, are accessible. Vectors may be needed in a delayed fashion after treatment to reach target cells that will migrate at the level of the clot or embolic agent. An ingenious approach was proposed to transfer genetic materials to the arterial wall with the use of stents carrying antibody-tethered adenovirus. To immobilize the adenovirus to the stent, anti-monoconal antibodies are covalently linked with the use of a biodegradable cross-linker. Recently adapted to coils to treat intracranial aneurysms, this method provides a way to retain at the target site therapeutic genes that could ultimately modulate the function of neointimal or endothelial cells that eventually migrate at the neck.

**Figure 1.** Neck of aneurysms showing hypothetical mechanisms involved in recurrences (d) or healing (c) after endovascular treatment of aneurysms. Therapeutic strategies to improve long-term results may include inhibition of recanalization or promotion of neointima formation.

**Figure 2.** Sequential steps involved in gene therapy of aneurysms. After selection of the gene of interest (step 1) and of the best promoter (step 2) and vector (step 3) for the application, the target cell must be determined for ex vivo gene therapy (step 4), followed by gene delivery (step 5). eNos indicates endothelial nitric oxide synthase; AAV, adeno-associated virus.
There may be other means to deal with this problem. We have explored the use of in situ beta radiation in endovascular treatment of aneurysms. Coils are made radioactive by ion implantation of $^{32}$P to prevent recanalization after embolization. It is possible to use beta radiation emitted from coils to specifically "turn on" gene expression at the neck of aneurysms with the help of radiation-inducible promoter/vector systems delivered by systemic or regional administration (Figure 3B).

**Target Cells for Gene Therapy**

To ensure efficacy of in vivo gene transfer strategies, gene delivery and gene expression must achieve significant levels. In some instances, the gene must be delivered to a specific cell type to reach the therapeutic objective. For example, to prevent thrombosis in nondenuded vessels, endothelial cells are the target. Conversely, medial smooth muscle cells may be targeted to prevent restenosis after balloon angioplasty. The endothelial lining is a biological barrier to viral vectors; angioplasty leads to denudation of the arterial wall, permitting viral vectors to reach smooth muscle cells effectively. In the future, vascular cells could be genetically modified to prevent aneurysmal development or to stabilize the aneurysmal wall in an effort to decrease risks of rupture. The cells that would be targets for gene transfer need not be the same as those that need to be modulated. Thus, endothelial cells could be transfected to overexpress a cytokine that, in turn, could promote collagen deposition by smooth muscle cells.

The target cells that should be targeted to promote healing after endovascular treatment of aneurysms have yet to be identified. Healing phenomena that promote the recovery of a permanent nonthrombogenic seal of the neck of aneurysms involve thrombus formation, migration and proliferation of VSMCs or myofibroblasts, synthesis of extracellular matrix, and reendothelialization. On the other hand, early endothelial migration and invasion of the clot may be responsible for recurrences. Consequently, enhanced healing after endovascular treatment may be achieved by inhibiting recanalization.
and/or promoting neointima formation at the neck of aneurysms (Figure 1).

A classic concept of phenotypic modulation of VSMCs responsible for neointima formation has been proposed\cite{31,52} and may apply to healing mechanisms after embolization.\cite{48} Cells harvested at the neck of embolized porcine aneurysms share similar characteristics with neointimal cells harvested from carotid arteries after angioplasty and respond in vitro to classic growth factors associated with neointima formation, such as platelet-derived growth factor (PDGF)-BB and transforming growth factor (TGF)-β.\cite{48,53} In vivo studies have shown that VSMC grafts increase neointima formation at the neck of canine aneurysms.\cite{57} VSMC grafts have exerted a protective effect against proteolysis in experimental aneurysm models.\cite{47} There is, however, no absolute evidence that VSMCs return to a pluripotent state, and the origin of neointimal cells remains to be clarified.

Cells recovered at the neck of treated aneurysms could be VSMCs, myofibroblasts, or circulating progenitor cells able to seed the thrombus and differentiate in response to given stimuli (eg, PDGF-BB). This last concept, proposed many years ago, has gained recent popularity.\cite{29,54}–\cite{57} Circulating progenitor cells may provide an alternative pathway to reach the site of embolization and express therapeutic genes. With this concept, circulating cells could be collected, cultured, and transfected, then reinserted into the host; these cells would reach the site of treatment, where the gene of interest could be locally expressed, and thus could potentially affect neighboring target cells. There is strong evidence that progenitors in the adult bone marrow can differentiate into tissues that overlap (eg, CD34 marker for early hematopoietic and angioblast lineages).\cite{54}–\cite{58} Progenitor cells can be harvested from peripheral blood or bone marrow and can be cultured and transduced ex vivo.\cite{29} Adult progenitor cells have the capacity, according to specific culture conditions, to differentiate into tissues that differ from the ones from which they originated. Unfortunately, several biological obstacles limit the use of progenitor cells for gene therapy.\cite{58}–\cite{60} They may be difficult to identify and isolate because cell surface markers of different lineages overlap (eg, CD34 marker for early hematopoietic and angioblast lineages). The pluripotent cells appear to be predominantly in G₀ phase of the cell cycle. When it becomes necessary to induce proliferation, differentiation has to be controlled. Finally, receptors for vector integration may be expressed at low levels.\cite{59}

**Methods to Transfer Genetic Material Into Cells**

Methods to introduce a gene into a cell include viral and nonviral vectors (liposomes, naked DNA). Viral vectors should meet several requirements: (1) high efficiency of infection; (2) stable replication of the foreign DNA either as an integrated transgene or as an episomal element; (3) appropriate and regulated expression in the target cells or tissues; and (4) adequate safety over time.\cite{8,29,60} Comprehensive reviews of vectoring methods and their relative merits can be found in several reports.\cite{8,29}–\cite{34} The nonviral methods rely on receptor-mediated endocytosis or fusion with the cell membrane. DNA delivered by nonviral methods is maintained in an extrachromosomal state and usually results in poor transfection efficiency and transient expression.\cite{31}–\cite{33} Nonviral techniques include transfection (calcium phosphate coprecipitation and electroporation), microinjection into the nucleus of target cells, particle bombardment with the use of a gene gun, cationic and polycationic liposomes, cationic lipids, virosomes (liposomes that contain viral proteins), and receptor-mediated gene delivery.\cite{61}–\cite{65}

Currently, adenoviral vector transfer remains the most commonly used method for in vivo gene transfer in cardiovascular applications.\cite{8,60,65} Adenoviral vectors can infect a broad range of cells, including VSMCs, endothelial cells, and vascular progenitor cells, and the efficiency of infection is 100-fold higher than plasmid DNA. However, in the absence of chromosomal integration, the genetic information is progressively lost with cell divisions. A major limitation is the host immune reaction triggered by residual viral antigens, leading to reduced transgene expression by elimination of infected cells and preclusion of a second vector administration.\cite{30}

Long-term expression into host cells requires the use of retroviral vectors that integrate genetic material into the chromosomal DNA of mitotic cells. Random integration into the host genome could, however, produce insertional mutagenesis, recombinations, and long-term cytotoxicity. Recent experiments have shown that recombinant Semliki Forest virus exhibits a high selectivity for VSMCs, leaving endothelial cells unaffected. The evanescent expression of this vector restricts its application, however.\cite{66}

To promote healing after endovascular treatment, transient gene expression may be sufficient, if not necessary, to avoid parent vessel stenosis from excessive intimal hyperplasia. In such a context, adenoviral vectors may be the appropriate vehicles.

**Cell- or Site-Specific Promoters**

Transgene expression with traditional promoters (such as cytomegalovirus or Rous sarcoma virus) affects many cells and tissues, but more specific targeting can be achieved with VSMC or endothelial-specific promoters.\cite{32,60,67} Examples of endothelial-specific promoters include thrombomodulin, von Willebrand factor, and Tie-2.\cite{32} Transcriptional induction by ionizing radiation can be accomplished by modification of transcription factors by a cellular kinase and binding of these factors to cognate elements in the promoter region of immediate-early genes such as EGR1.\cite{67} Thus, another means of targeting transgene expression to the tissues developing on embolic devices is to use radiation-sensitive promoters in combination with beta-emitting coils.\cite{43,44,68}

**Candidate Genes to Improve Results of Endovascular Treatment of Aneurysms**

Once the delivery method has been developed or refined, any gene potentially involved in vascular remodeling may be considered to alter the evolution of intracranial aneurysms; only a few will be mentioned here.

Genes that have been linked to cell migration, proliferation, matrix production, or degradation, such as growth
factors, have been first candidates. For example, TGF-β regulates a broad range of biological activities related to neointima formation and recovery after vascular injury. TGF-β induces VSMC migration and synthesis of extracellular matrix. TGF-β may stabilize experimental aneurysms. Thus, TGF-β also inhibits proteolysis and endothelial cell migration. Thus, TGF-β could be locally expressed to inhibit recanalization and favor neointima formation at the neck of treated aneurysms. Unfortunately, there was no added benefit from recombinant TGF-β overexpression on healing of canine aneurysms compared with cellular grafts expressing a marker gene only.

Other growth factors, such as PDGF-BB, basic fibroblast growth factor (bFGF), and VEGF have also been considered for cardiovascular applications.

Because recanalization after coil embolization is linked to endothelial invasion of the clot, antiangiogenic strategies could be used to improve results after endovascular treatment. Decreased reendothelialization has been associated with increased neointima formation and can be achieved with an adenosine expressing endostatin. This approach could be combined with in situ beta radiation.

The potential use of other genes derives from experimental observations. For example, there is a relative deficiency of collagen type III and a reduction in the elastin/collagen ratio in aneurysms compared with the normal arterial wall, possibly related to accelerated enzymatic degradation by MMPs. The main metalloproteinase found in small aneurysms is MMP-2, but MMP-9 may be involved in aneurysm expansion at a later stage. Overexpression of inhibitors such as tissue inhibitor of metalloproteinase (TIMP) or plasminogen activator inhibitor (PAI) has been shown to improve the evolution of experimental abdominal aneurysms. Other investigators have explored the role of nitric oxide and nitric oxide synthase in aneurysm formation and found upregulation of genes involved in oxidative stress (e.g., heme oxygenase, inducible nitric oxide synthase) and downregulation of antioxidant genes (e.g., superoxide dismutase). These genes offer alternative pathways to modulate biological phenomena at the neck of treated aneurysms.

DNA arrays are new methods of exploring transcript profiles in molecular biology. The differential expression pattern of a particular gene (upregulation or downregulation) can be observed in normal and pathological states. Gene expression patterns of growth factors, adhesion molecules, extracellular proteins, MMPs, and their inhibitors, related to chronic inflammation, extracellular matrix degradation, and smooth muscle cell depletion found in aneurysms, are being discovered. These large amounts of data obtained by these techniques do not necessarily help to identify key factors or most pertinent genes. The altered gene expression found in aneurysms may still add new insights regarding their biological evolution. Comparisons of genes expressed with recanalization after coil occlusion or with aneurysmal recurrence in experimental models are areas in which this approach could provide therapeutic avenues. Once a gene is suspected of being involved in aneurysm formation or progression, knockout studies, as suggested by Carmeliet et al., may confirm its potential protective or participating contribution to the evolution of aneurysm. Another possibility is to introduce the gene of interest into vascular smooth muscle cells grafted into a xenograft rejection model, as described by Allaire et al. A similar method, using smooth muscle cell grafts in a lateral wall canine aneurysm model embolized with collagen sponges, was not as helpful because the therapeutic effects of cell grafting per se precluded the study of the effects of the gene of interest carried by transplanted cells.

Conclusions

Gene therapy may provide a future means of improving results of endovascular treatment of aneurysms. Multiple technical and conceptual obstacles remain to be addressed, however, before this technology becomes a realistic option in clinical practice.

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