Editorial

Gene Therapy of Restenosis
Promise and Perils

Peter Libby

The advent of technology for vascular gene transfer some 8 years ago led to an explosion of interest in the notion that local gene therapy could modify the natural history of arterial hyperplastic diseases. What better testing ground for this concept than restenosis following percutaneous transluminal coronary angioplasty (PTCA) or other arterial interventions? In this situation, the precise moment of onset of the pathological process, an iatrogenic arterial injury, is known. Furthermore, the site of the intervention predicts exactly where subsequent pathologies will develop. Finally, this very segment of the vasculature is instrumented at the time the pathological process commences.

A host of promising studies in animals has fueled enthusiasm for treatment of human restenosis by gene therapy. Various strategies, ranging from inhibition of genes involved in the cell cycle with antimetabolites to overexpression of nitric oxide synthase, have yielded reductions in intimal thickening after injury in experimental animals. The now widespread implantation of stents in diseased arteries provides further opportunity for transfer of nucleic acids or even genetically modified cells.

In view of these advances, are we at the threshold of a halcyon era of vascular intervention, or do obstacles remain that may frustrate the rapid application of these existing advances to patients? This issue is debated by the point-counterpoint articles in this issue (DeYoung & Dichek; Baek & March). This editorial aims to highlight the importance of this debate and also emphasize some potential problems that require consideration as application of recent advances in gene transfer progresses toward clinical application. Notably, successful application of gene therapy to human restenosis presupposes that (1) we know which gene to transfer and (2) we possess the technology to do so efficiently and without causing harm.

Intimal “Proliferation”: An Appropriate Target for Gene Therapy in Human Restenosis?

Much of the experimental work on the treatment of restenosis has built on a solid basis of data emerging from studies of injury to previously normal animal arteries. These studies have no doubt advanced our understanding of the artery’s response to injury in an enduring and fundamental fashion. Yet, recent results from both animal and human studies suggest that the reduction in caliber encountered in restenosis may not depend as much on intimal “proliferation” as simple injury models that have proven so instructive from a biological perspective.

Does Intimal Thickening Cause Luminal Narrowing in Restenosis?

A number of animal studies have called into question the primordial role of intimal thickening in this regard. Kakuta et al in Faxon’s laboratory found that shrinkage of the entire vessel, judging from a reduced area encompassed by the external elastic lamina, distinguished angiographically restenotic versus nonrestenotic arteries in a doubly-injured artery. They did not find a correlation between angiographic restenosis and intimal thickening in this study. Lafont et al found in a similar preparation that angiographic restenosis correlated with what they termed a “chronic constriction index” rather than with intimal thickening. Recent clinical studies evaluating the structure of the artery wall in addition to luminal diameter as determined by angiography have come to much the same conclusion. Failure to maintain expansion of the dilatation of the artery wall produced by intervention rather than intimal thickening determined by intravascular ultrasound occurred after injury. Similar data are currently emerging from other centers as well. In sum, it appears less and less clear that intimal thickening holds the sole key to human restenosis.

Is Smooth Muscle Cell Replication a Sensible Target in Human Restenosis?

Smooth muscle cell migration and division doubtlessly contribute importantly to intimal thickening following injury to a previously normal artery. However, abnormal arteries, already exhibiting a complex intimal lesion, represent the substrate for arterial interventional therapy in humans. Evidence for ongoing smooth muscle cell division in these arteries is relatively weak. Pickering et al showed relatively high indices of proliferation in specimens of human restenotic lesions retrieved by atherectomy. They assessed cell division by expression of the proliferating cell nuclear antigens (PCNA). The sample specimens studied by Pickering et al included many specimens obtained from restenotic peripheral vessels. The study of O’Brien et al, using a larger number of restenotic coronary artery atherectomy specimens, showed negligible evidence for smooth muscle cell proliferation in the vast majority of cases. These investigators also presented substantive controls indicating the specificity of the PCNA method for detecting cell
proliferation under the conditions of their study. It could be argued that cell proliferation occurred early on in the restenotic process and that the low rates of replication evident from study of the atherectomy specimens merely reflected the delay between an early phase of cell division and the time at which the specimen was retrieved. In O’Brien’s series, even those specimens obtained at earlier times after intervention failed to disclose evidence for ongoing smooth muscle cell proliferation.

Even if O’Brien et al missed a possible early “wave” of smooth muscle cell replication, as already discussed, mechanisms other than smooth muscle proliferation could lead to intimal thickening. For example, the bulk of the volume of restenotic lesions appears composed of extracellular matrix rather than cells themselves.18,19 Thus, intimal extension could occur merely by augmented extracellular matrix elaboration by resident smooth muscle cells, without invoking a high degree of vascular smooth muscle cell proliferation.

However, for the purposes of argument, let us stipulate that smooth muscle cell division does indeed determine lumen loss in human restenosis. Could inhibition of smooth muscle cell replication adversely affect healing of an injured artery and adversely affect outcome in this way or others? The smooth muscle cell elaborates the extracellular matrix, which is required for arterial repair following injury. Effective inhibition to smooth muscle cell division or elimination of smooth muscle cells might lead to aneurysm formation. This concern applies to local radiation therapy of restenosis as well as genetic manipulation targeting smooth muscle cell replication. Moreover, elimination of smooth muscle cells or prevention of their replication might yield a weakening of the fibrous skeleton of the target lesion.20,21

We now recognize that disruption of atheromatous plaques underlies the thrombus that causes most acute coronary syndromes. One pathway of benefit of PTCA in a subset of unstable lesions might be controlled disruption with a short-term anticoagulant treatment sufficient to limit acute thrombosis. The iatrogenic plaque disruption, repaired by a healing process contributed to by smooth muscle collagen synthesis, could reinforce the fragile fibrous cap that led to the unstable coronary syndrome in the first place.22 Indeed, patients with restenosis seldom present with acute myocardial infarction. This observation suggests that lesions post PTCA are less prone to provoke thrombosis than stenosing lesions that occur in arteries that have not undergone intervention. For this reason, successful inhibition of smooth muscle cell replication might actually interfere with one of the mechanisms of benefit of PTCA, one that might contribute to the success of coronary interventional treatments in the majority of cases that do not “restenose.”

Consider also the potential extension of this argument to the case of systemic therapy targeting smooth muscle cell replication. Weakening of the fibrous cap can render an atheromatous plaque vulnerable to disruption, thrombosis, and production of an acute coronary syndrome. For every coronary artery stenosis sufficient to warrant angioplasty, the same patient’s coronary tree probably contains several other clinically silent, perhaps angiographically modest-appearing lesions. Impairment of smooth muscle cell function in the neighboring atheroma might favor their destabilization. In this manner, a systemic antiproliferative treatment might actually increase “coronary events,” even if they succeeded in inhibiting restenosis of the target lesion.

**Current Gene Transfer Vectors: Ready for Clinical Application?**

A number of different strategies for gene therapy are under consideration. Adenovirus-mediated gene transfer figures prominently among techniques currently contemplated for clinical use. Successful implementation of this strategy will require considerable effort to control the site of delivery, the efficiency of infection, the amount of gene product that will be expressed, and the duration of expression and persistence of the protein product of the transferred gene. No standard pharmacological therapy would enter the clinic without thorough evaluation of issues such as dose, timing of the doses, pharmacokinetics, absorption, and concentration at the target tissue. In the case of virally mediated gene transfer, we still lack a great deal of important information along these lines.

Another issue that warrants serious consideration in drug development, and should receive equal attention in the context of gene therapy, is the potential of unwanted effects. Retroviral gene transfer, despite a considerable effort in engineering of the vectors, still raises the possible concern of insertional mutagenesis or acquisition by complementation of replicative or transforming potential. In the case of adenoviral vectors, issues regarding potential inflammatory effects of these infectious particles persist. The “extinction” of adenoviral gene expression in the weeks following infection seems to depend on the cellular immune response, indicating that the adenoviral vectors retain proinflammatory effects. Indeed, Newman et al22 have demonstrated that adenoviral infection itself can provoke arterial inflammation. The arterial pathology produced by adenoviral infection includes prominent infiltrates of T lymphocytes and activation of some of the very same functions of vascular wall cells associated with the early atherogenesis, such as increased expression of adhesion molecules including intracellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1). Moreover, these studies revealed that adenoviral infections by themselves could provoke intimal thickening in infected arteries. Thus, present-day adenoviral vectors may actually produce considerable vascular pathological responses themselves, including intimal thickening, a process often itself the target of strategies to prevent restenosis by gene therapy.

**Conclusions**

The promise of gene therapy in the treatment of arterial and many other diseases remains bright. However, glossing over the potential problems encountered in the early phases of development of this technology may prove deleterious to its further advance. Both point/counterpoint articles in this issue discuss these issues in depth (DeYoung & Dichek3; Baek & March4). In addition, this editorial and the two point/counterpoint articles also suggest potential novel areas of investigation to further the development of gene therapy.

The scientific community needs to avoid raising prematurely public expectations for rapid clinical applications. Similarly, the enthusiasm of public and private sector funding...
sources for development of emerging therapeutic strategies may prove short-lived in the face of repeated failures to realize heightened expectations. Equally important, premature application of gene therapy in the clinic, without sufficient attention to some of the problems enumerated above, might lead to an adverse outcome in a clinical trial. Such an occurrence would undoubtedly raise the threshold of regulatory agencies, ultimately inhibiting the development of this promising field.

Our goal in gene therapy of restenosis should be to master the biology sufficiently so that we can identify an appropriate pathological process to target this gene therapy. We also need to consider the potential deleterious effects of gene transfer, and we need to take into account the possibility that currently available vectors may produce adverse effects that could mitigate the desired therapeutic action.

Although our short-term outlook on the application of gene therapy to human restenosis must be tempered by the foregoing considerations, the longer-term future remains quite bright. Progress in the design of vectors will likely render them less immunogenic. Such modifications should prevent some of the proinflammatory effects documented, for example, with current adenoviral vectors. Interestingly, this very approach to reducing immune-mediated inflammation may permit longer persistence of the virus and enhance the duration of action of the transferred gene. With regard to identifying appropriate targets for gene therapy, the explosion in vascular biology of restenosis and an increasing appreciation of the complexity of human restenosis will no doubt bear fruit by identifying targets for gene therapy applicable to human restenosis. Even more encouraging, a true mastery of the biology of the blood vessel walls might permit us to prevent formation of the lesions targeted by vascular intervention rendering this halfway technology as anachronistic as producing pneumothoraces to treat tuberculosis.

References


Key Words: gene therapy • restenosis • vectors
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Circ Res. 1998;82:404-406
doi: 10.1161/01.RES.82.3.404

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
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