New Perspectives in Hypertension Research
Potentials of Vascular Biology

Victor J. Dzau, Gary H. Gibbons, Ryuichi Morishita, Richard E. Pratt

Abstract The vessel wall was once considered to be a passive conduit responding to the circulating endocrine system. However, the emergence of molecular and vascular biology in hypertension research has redefined our understanding of the role of the vasculature as a vital organ in the pathogenesis of hypertension. It is now recognized that the vasculature can regulate its own tone by a variety of previously unknown autocrine and/or paracrine vasoactive systems. Recent evidence indicates that the process of vascular remodeling in hypertension appears to be mediated by locally generated factors within the vessel wall. This review examines the implications of this new paradigm in hypertension, focusing on five topics that have developed through the emergence of molecular vascular biology: the discovery and characterization of novel biologically active molecules synthesized by the vessel wall, the molecular mechanisms and consequences of vascular remodeling, the developmental biology of the blood vessel and the relation to pathobiology, the use of in vivo gene transfer to test hypotheses in vivo, and novel treatment strategies based on gene therapy of the vessel wall. (Hypertension. 1994;23 [part 2]:1132-1140.)

Key Words • muscle, smooth, vascular • transfection • gene therapy

Over the past half century, the direction of hypertension research has been shaped by a scientific paradigm that has focused on the regulation of systemic neuroendocrine vasoactive systems that control vascular tone and renal fluid-electrolyte homeostasis. This paradigm postulates that hypertension is caused by a disturbance in the physiological homeostatic mechanisms regulating the levels of and/or responses to circulating hormones and sympathetic nervous system activity. In this schema the vasculature is conceptualized as a simple effector system that passively responds to the actions of systemic neuroendocrine factors. However, advances in molecular vascular biology have begun to promote a paradigm shift that has dramatically altered our conception of the role of the vasculature in hypertension. Based on recent studies it has become increasingly clear that the vasculature is itself a complex, integrated organ capable of autonomous generation of locally active factors that mediate changes in tone and structure. This review will examine the implications of this new molecular vascular biology paradigm in hypertension research, focusing on five areas in which vascular biology has had or will have major effects on hypertension research: (1) the discovery of novel endogenous biologically active molecules synthesized by the vessel walls (2) the elucidation of molecular mechanisms and consequences of vascular remodeling in hypertension, (3) an understanding of the developmental biology of the blood vessel and the relation to pathobiology, (4) the in vivo testing of in vitro hypotheses using in vivo gene transfer, and (5) the development of novel treatment strategies based on vascular molecular biology, such as gene therapy.

From the Falk Cardiovascular Research Center, Division of Cardiovascular Medicine, Stanford (Calif) University School of Medicine.

Correspondence to Victor J. Dzau, MD, Falk Cardiovascular Research Center, Division of Cardiovascular Medicine, 300 Pasteur Dr, Stanford, CA 94305-5246.
data indicate that the regulation of nitric oxide generation by the endothelium may be a critical determinant of blood pressure in hypertension.

Advances in vascular biology have also reshaped our understanding of previously characterized classic hormonal systems. For example, it is now recognized that a paracrine vascular natriuretic peptide system exists in addition to the classic endocrine system. It is speculated that the recently discovered endothelin-derived type C natriuretic peptide may be an important paracrine system regulating vascular tone and structure. Similarly, the endocrine renin-angiotensin system has been recognized for nearly 100 years, yet the importance of an autocrine-paracrine vascular angiotensin system has been appreciated only within the past decade. The technologies of molecular biology have defined the components of the enzymatic cascade necessary for the local generation of angiotensin II (Ang II) within various tissues, including the vasculature.

Endothelin-1 is yet another example of a vasoactive molecule that was previously unknown little more than a decade ago. Its potency and long-lived vasoconstrictive effects fueled speculation that it may be an important mediator of hypertension. With the recent emergence of specific endothelin-1 antagonists, we anticipate that the role of endothelin-1 in hypertension will be further characterized. We speculate that similar to the experience with other vascular paracrine systems, the measurement of plasma levels will underestimate the role of endothelin-1 in the pathogenesis of various vascular disorders. Other vessel wall paracrine systems include the kallikrein-kinin system, prostanoids, and endothelin-derived hyperpolarizing factors. Moreover, the vascular biology paradigm would predict that the effects of vasoactive substances such as endothelin on vascular cell growth and vessel structure may be as important in the pathogenesis of hypertension as the effects on vessel tone.

In association with this expanding list of autocrine-paracrine mediators is a growing appreciation of their pleiotropic effects on cell function. It is now clear that vasoactive substances modulate cell growth, migration, matrix production and degradation, thrombosis, and leukocyte adhesion in addition to their effects on vascular tone. These multifunctional properties may partly reflect the multiplicity of cellular receptors and signaling pathways activated by these vasoactive agents. It is of interest that there are three types of natriuretic peptide receptors (A, B, and C), at least three types of angiotensin receptors (AT1A, AT1B, and AT2) and at least two types of endothelin receptors (ET1A and ET1B). It is intriguing that the expression of these receptors is altered by changes in cell differentiation or phenotype that occur during ontogeny or in response to vascular injury. We speculate that the regulation of vasoactive peptide receptor expression may be an important factor in the pathogenesis of vascular diseases.

Based on exciting new evidence we can now conceptualize the vasculature as an integrated vital organ capable of sensing environmental cues and producing appropriate biologic mediators to modulate tone and structure. We anticipate that further investigation of the vascular biology of autocrine-paracrine factors in hypertension will provide important new insights into the pathogenesis and treatment of this disease.

### Table 1. Spectrum of Vascular Remodeling

<table>
<thead>
<tr>
<th>Stimulus</th>
<th>Clinical Example</th>
<th>Vascular Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>↑ Pressure</td>
<td>Chronic hypertension</td>
<td>(a) ↑ Wall thickness, ↓ lumen diameter</td>
</tr>
<tr>
<td>↑ Flow</td>
<td>Arteriovenous fistula</td>
<td>↓ Inner diameter, ↑ outer diameter</td>
</tr>
<tr>
<td>↓ Flow</td>
<td>After stenosis</td>
<td>Myointimal hyperplasia</td>
</tr>
<tr>
<td>Injury</td>
<td>Balloon angioplasty</td>
<td>Atherosclerosis</td>
</tr>
</tbody>
</table>

LDL indicates low-density lipoprotein.
hypothesized that most endogenous vasoconstrictive substances are also growth promoters and most endogenous vasodilator substances are growth inhibitors.32, 33 Accordingly, vasoactive substances can regulate vascular homeostasis via its short-term effects on vascular tone and its long-term effects on vascular structure. In hypertension, an imbalance of these factors favors vasoconstriction and vascular hypertrophy.

Table 1 summarizes the broad spectrum of geometric and structural alterations of the blood vessel. A blood vessel can achieve an increase in mass, leading to a thickening of the wall (eg, in hypertension). In this case, the intimal and/or medial layers may increase in size and the inner diameter decrease. Consequently, the ratio of diameters of the wall to lumen increases. Hypertensive vascular hypertrophy resulting from an increase in smooth muscle mass may amplify the vasoconstrictive response to neurohormones and may perpetuate hypertension by accentuating systemic vascular resistance. These changes within hypertensive vessels are seen primarily in small arteries and arterioles, although the large vessels also undergo remodeling involving cellular hypertrophy and matrix changes.34, 35 In these large vessels the functional changes are primarily expressed as decreases in compliance and distensibility. An increased wall-to-lumen ratio with encroachment of the lumen may also occur in hypertensive resistance vessels without the increase in medial smooth muscle cell mass as described by Mulvany,34 Baumbach and Heistad,36 and others. This is accomplished primarily by a rearrangement of the smooth muscle and other cellular and noncellular elements, resulting in a primary reduction of the diameter.

Another form of remodeling relevant to hypertension is that of enlargement or dilation of the blood vessel. This is the result of active restructuring of the cellular and noncellular components of the vessel wall. The total mass of the blood vessel may not increase. However, both the outer and inner diameters increase substantially, resulting in a reduced ratio of wall to lumen diameter. This form of remodeling is usually seen in large vessels. An example of this form of remodeling is vascular enlargement associated with a sustained high blood flow.37 Finally, rarefaction of the microcirculation (a loss of capillary area), which is reported to occur in hypertension, may also be considered as a form of remodeling.38 An understanding of the mechanism of vascular remodeling may alter our therapeutic strategy in hypertension.

The spectrum of signals ranges from mechanical forces (shear stress and stretch) to vasoactive substances and inflammatory mediators. A number of reports have documented that shear stress may play an important role in regulating the size and structural characteristics of the vessel wall. Flow-induced vascular remodeling depends on the presence of an intact endothelium39 and is consistent with the concept of shear stress autoregulation, in which the vessel remolds itself such that the lumen is reshaped to maintain a given level of shear stress.39 Endothelial cells apparently sense shear stress via the activation of an inwardly rectifying potassium channel and hyperpolarization.40, 41 Moreover, shear stress can activate phosphoinositols metabolism and may promote an increase in intracellular calcium.42, 43 These events result in the release of local factors, such as nitric oxide, transforming growth factor-β (TGF-β), platelet-derived growth factor (PDGF), tissue plasminogen activator, and endothelin, that may participate in vascular remodeling.44-47 The blood vessel is also exposed to biaxial tension: tension in the plane of the endothelial and smooth muscle cells and compression along the radial plane. Recently, Harder48 reported that the autoregulatory increase in tone in response to stretch in the cerebral vasculature is caused by the release of an endotheium-dependent humoral factor or factors. The effect of stretch may also modulate vascular structure.49 The signal transduction mechanisms mediating the effect of stretch on vascular function are currently under study. One possible mechanism is suggested by the recent discovery by Lansman et al50 of a stretch-responsive nonspecific ion channel in bovine aortic endothelium. In addition to hemodynamic factors, the endothelium responds to a variety of stimuli by specific receptor-coupled events. The calcium ion appears to play an important role in the signal transduction function of the endothelium. The changes in intracellular calcium by humoral factors are mediated via activation of phosphoinositides52-55 or receptor-coupled ion channels or agonist activation of potassium channels in endothelial cells.56-57 In response to hemodynamic forces, physical injury, or circulating factors, the cells in the vessel wall become activated to release growth modulators, cytokines, proteolytic enzymes, and matrix components, thereby participating in the process of vascular remodeling. Table 2 lists the autocrine-paracrine factors that regulate vascular cellular growth. These factors can be divided into growth promoters, growth inhibitors, or vasoactive substances with growth-regulatory properties. This classification is not rigid because it has been shown that a single factor may manifest multiple properties and perform several functions depending on the experimental condition.56-61

In addition to changes in cell migration and proliferation, matrix modulation is a key event in vascular remodeling. Expansion or contraction of extracellular matrix is a dynamic process that involves synthesis and/or degradation of matrix proteins and carbohydrates, including glucosaminoglycans, hyaluronic acid, collagen, elastin, and other components. Proteolysis and matrix degradation involve the activation and action of endogenous proteases such as tissue plasminogen activator, plasmin, elastase, collagenase, hyaluronidase, and haptoglobin.52-65 Endothelial cells also express a variety of adhesion molecules that regulate their adhesion to extracellular matrix and the organization of cell-cell junction.56, 67

In hypertension, alterations in vascular structure are probably the consequence of increased pressure and flow, an imbalance of vasoactive substances, and endothelial dysfunction. Investigations of the pathobiology of hypertension and vascular remodeling are being
and contain low quantities of rough endoplasmic reticulum and a highly ordered contractile apparatus. Studies at the molecular level suggest that the most striking differences exist in the expression of contractile proteins and extracellular matrix. For example, myosin heavy chain exists as at least three isoforms, each exhibiting differential expression during prenatal and postnatal development. Extracellular components such as fibronectin, osteopontin, tropoelastin, and procollagen also exhibit high levels of expression in the neonate but not the adult vessel. In addition, growth factors and their receptors also exhibit differential expression.

In many instances, the response of the vascular smooth muscle cells (VSMCs) to injury involves a reexpression of this neonatal pattern of genetic expression. This has been examined in the greatest detail in the balloon-injured rat carotid artery, which develops neointimal hyperplasia. Many of the neonatal markers that are reexpressed in the growing VSMCs are constituents of the contractile apparatus, the paracrine growth factors, or the extracellular matrix and are presumably important to the growth characteristics of the cells. The reexpression of developmentally regulated genes in the vessel wall in response to injury may have several implications toward the molecular mechanism of neointimal formation. The data are consistent with two distinct hypotheses. One hypothesis involves the dedifferentiation of the mature medial smooth muscle cells in response to injury. This dedifferentiated cell undergoes proliferation and migration to populate the neointima. The second hypothesis involves the presence within the normal media of an undifferentiated "stem" cell that responds to vascular injury with proliferation and migration. In either situation, some of the common biologically active substances synthesized in the developing blood vessel and the diseased adult blood vessel may play important roles in vascular cell growth, migration, and differentiation. Indeed, several growth factors (e.g., PDGF, basic fibroblast growth factor, and TGF-β) have been shown to be expressed in the developing blood vessel and injured adult blood vessel.

In the context of hypertension, the potential expression of the angiotensin system in the vessel wall during development and in neointimal hyperplasia is particularly interesting. ACE is expressed by the endothelium of adult blood vessels. It is barely detectable, if at all, in the adult tunica media or adventitia. However, in the neonate ACE is expressed in high concentration in the tunica media of the aorta. In the 1-day-old rat the tunica media of the aorta exhibits intensive ACE staining in VSMCs throughout the entire layer. The staining is dramatically reduced in the 3-day-old vessel. By days 7 and 14, the staining pattern is similar to that observed in the normal adult vessel, with staining restricted primarily to the endothelial cells. ACE expression in the neonatal vessels persists in cell culture. Smooth muscle cells isolated from neonatal vessels express ACE at levels fourfold to fivefold higher than seen in adult vessels. The presence of ACE within the developing vessel is suggestive of a role for Ang II in the developmental process. It is well known that Ang II exerts a growth-promoting effect on VSMCs both in vivo and in vitro via the autocrine induction of several growth factors, such as

<table>
<thead>
<tr>
<th>Table 2. Vascular Smooth Muscle Cell Growth Modulators</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth Promoters</td>
</tr>
<tr>
<td>------------------</td>
</tr>
<tr>
<td>aFGF, bFGF</td>
</tr>
<tr>
<td>PDGF-AA, AB, BB</td>
</tr>
<tr>
<td>TGF-β, 1, 2</td>
</tr>
<tr>
<td>HB-EGF</td>
</tr>
<tr>
<td>EGF</td>
</tr>
<tr>
<td>IGF-1</td>
</tr>
<tr>
<td>IL-1, IL-6</td>
</tr>
<tr>
<td>Thrombin</td>
</tr>
<tr>
<td>Serotonin</td>
</tr>
<tr>
<td>Angiotensin II</td>
</tr>
<tr>
<td>Endothelin</td>
</tr>
<tr>
<td>Norepinephrine</td>
</tr>
<tr>
<td>Vasopressin</td>
</tr>
<tr>
<td>Substance P, K</td>
</tr>
<tr>
<td>Leukotrienes</td>
</tr>
<tr>
<td>Thromboxane</td>
</tr>
<tr>
<td>Stretch-wall tension</td>
</tr>
<tr>
<td>LDL</td>
</tr>
</tbody>
</table>

aFGF indicates fibroblast growth factor; bFGF, basic FGF; PDGF, platelet-derived growth factor; TGF, transforming growth factor; HB-EGF, epidermal growth factor; IGF, insulin-like growth factor; IL, interleukin; and LDL, low-density lipoprotein.

*Bi-functional growth properties.
PDGF, TGF-β, and basic fibroblast growth factor. These growth factors can affect not only the proliferation and migration of smooth muscle cells in an autocrine fashion but can also stimulate surrounding vascular cells such as the endothelium and fibroblasts. Although it is highly likely that a role exists for the tissue Ang II system in the normal developmental process, delineation of this role remains to be elucidated.

Consistent with the reexpression of a neonatal phenotype after injury, ACE activities in the rat aorta and carotid artery after balloon injury are significantly increased. Immunohistochemical analysis shows that in normal blood vessels only endothelial cells stain positively for ACE. In the balloon-injured aorta, the neointimal VSMCs stain intensely with the ACE antibody. These data are consistent with the hypothesis that neointimal smooth muscle cells, similar to those from the neonatal vessel, express ACE, which results in increased local Ang II production and contributes to the development of the neointima. This hypothesis is supported by the data of Powell et al, who reported that ACE inhibitors can prevent neointimal proliferation after balloon injury in rats. This effect of ACE inhibitors on experimental restenosis is in part due to the blockade of Ang II production, because the angiotensin receptor antagonist DuP 753 can also blunt neointimal hyperplasia.

Another component of the renin-angiotensin system that is uniquely expressed in the developing blood vessel is the type 2 Ang II receptor (AT2 receptor). In the adult blood vessel and most tissues, the predominant Ang II receptor is a seven-transmembrane G protein-coupled receptor (AT1 receptor) that either activates phospholipase C or inhibits adenylate cyclase. A second receptor isoform (AT2 receptor), defined by its selective binding to the ligands PD 123319 and CGP 42112, has been described but no function has been identified. Interestingly, this receptor is highly expressed in embryonic vascular tissue but only at low levels, if at all, in those of the adult. Radioligand binding experiments performed on membranes prepared from whole injured and uninjured carotid arteries showed that only AT1 is expressed in the uninjured vessels. In the injured carotid, both AT1 and AT2 bindings are observed. These data demonstrate a partial "switch" of the angiotensin receptor in the injured vessel from the AT1 to the AT2 isof orm. With the use of in vitro autoradiography, our preliminary data show that the AT2 receptor is localized primarily to the neointimal VSMCs, consistent with the observation of ACE expression in the neointima and neonatal VSMCs. What is the role of the Ang II receptor isoforms during the vascular response to injury? The cellular action of AT2 receptor activation is unknown. The neonatal expression of the AT2 receptor has led to the speculation that this receptor may be involved in the growth of VSMCs during development and in response to injury.

These studies on the reexpression of a neonatal phenotype in the adult diseased vasculature have primarily used the balloon-injured rat carotid artery as a model. What then is the significance toward an understanding of the biology of the hypertensive vessel? Schwartz et al have shown that the hypertensive vessel contains a higher proportion of the "synthetic" phenotype compared with the nonhypertensive vessel. Changes in extracellular matrixes have been noted in hypertensive vessels, notably the expression of fibronectin. Alternatively spliced forms of fibronectin exist and exhibit differential expression during development. Takasi et al have shown that a particular splice variant, EIIIA, is induced in experimental hypertension. Interestingly, this variant is also expressed at high levels in the neonatal but not the normal adult aorta. These investigators have also demonstrated the induction of TGF-β1 in the hypertensive vessel. TGF-β1, a factor that plays a causal role in vascular remodeling, is expressed at high levels in the injured vessel but not in the normal adult vessel. The developmental expression in the vessel wall has not been reported; however, this factor is highly expressed in neonatal tissues, such as the heart. In the context of our observation on vascular angiotensin, it is interesting to note that ACE expression, which occurs at high levels in neonatal vessels, is increased in the aorta of rats with two-kidney, one clip hypertension. Interestingly, treatment of young prehypertensive spontaneously hypertensive rats (SHR) with converting enzyme inhibitors will protect the animals from posttreatment increases in blood pressure. It has been hypothesized that alterations in the vessel wall might amplify and perpetuate hypertensive signals, perhaps indicating that in the SHR vasculature an ACE-dependent remodeling is occurring in the prehypertensive state. Consistent with this, the SHR vessel wall has been reported to undergo alterations before the development of hypertension. To date, no studies of AT1 versus AT2 receptor expression in hypertensive blood vessels have been reported. Interestingly, Rogg et al detected AT1 binding in the myocardium of patients with increased atrial pressure but not in the normal myocardium. Taken together, these data suggest that an understanding of the developmental biology of the blood vessel can provide important insight into the pathobiology of vascular disease, including hypertension.

In Vivo Test of In Vitro Hypothesis: A Gene Transfer Approach

Many substances synthesized in the vasculature have been postulated to exert autocrine-paracrine actions on vascular function and/or structure (Table 2). However, definitive proof for their functions cannot be obtained because in vivo studies are limited by the multiplicity of coexisting variables, the difficulties in manipulating individual components, and the methodological limitations in studying the function of locally produced modulators in the absence of any contribution by the circulation. Cell culture and gene transfer technologies have provided us with the opportunity to study cellular responses to the manipulation of the individual components of a putative autocrine-paracrine mediator system (i.e., by overexpression or inhibition). For example, transfection of an ACE expression vector into cultured VSMCs resulted in increased cellular ACE activity and stimulated DNA and RNA synthesis via increased Ang II production. These findings indicate that VSMCs contain components of the renin-angiotensin system and that cellular ACE is a rate-limiting step in Ang II generation in VSMCs in vitro. An in vivo experimental approach that can selectively manipulate vascular wall gene expression would be useful for furthering the understanding of the biology and pathobiology of the autocrine-paracrine system.
Recent progress in in vivo gene transfer technology may provide us with this possibility. The feasibility of transfecting blood vessels with foreign DNAs in vivo was shown previously with the use of retroviral vectors. As an alternative, direct physical transfer of genes into intact blood vessels in vivo has been reported with the use of cationic liposomes (Lipofectin) as the DNA carrier. Recently, the adenoviral vector transfer method has also been used successfully for gene transfer into the blood vessel. Several functional genes have been successfully transferred into endothelial cells and VSMCs in vivo. For example, the transfection of PDGF gene induced VSMC proliferation, whereas the transfection of fibroblast growth factor gene caused both angiogenesis as well as VSMC accumulation. These results emphasize the potential utility of the in vivo gene transfer method to dissect out the biologic role of locally generated vasomodulators within the vessel wall in vivo.

To further define the biologic role of locally generated vasoactive factors, we have recently used hemagglutinating virus of Japan (HVJ)-liposome-mediated gene transfer into the vascular wall. This method yields high transfection efficiency (up to 30% of vessel wall cells may be transfected) without apparent toxicity. The usefulness of HVJ-liposome gene transfer for in vivo vascular biology studies has permitted us to examine the question of the autocrine-paracrine system in the vessel wall. The role of vascular ACE as a determinant of vessel tone and structure is a controversial subject in hypertension research. Given the difficulties of distinguishing between circulating and vascular ACE effects, traditional experimental approaches using pharmacologic tools have provided only indirect evidence of the potential importance of vascular ACE as a determinant of local Ang II generation. Using in vivo gene transfer, we tested the hypothesis that increased local expression of vascular ACE and production of Ang II are important determinants of vascular structure. Our data demonstrate that increased local expression of ACE within the vessel wall promotes autocrine-paracrine Ang II-mediated vascular hypertrophy in vivo. Thus, the use of in vivo gene transfer enabled us to address two important questions in hypertension and vascular research: (1) Can angiotensin directly mediate vascular hypertrophy independently of its blood pressure effect? and (2) is local tissue ACE important in regulating local vascular function and contributing to pathophysiology? These results have important implications in our understanding of the role of locally generated vasoactive substances in the process of vascular remodeling in hypertension.

As described above, in vivo gene transfer is suitable for studying vascular remodeling by local overexpression or inhibition of vasomodulators. In vivo gene transfer technology has the following advantages over conventional experimental approaches such as classic pharmacology: (1) The target gene can be transfected into a local segment of a blood vessel, thereby avoiding a systemic effect; (2) this transfected vascular segment can be compared with adjacent untransfected segments or with the contralateral control blood vessel, which are subject to the same hemodynamics and circulating humoral factors; and (3) the consequences of local overexpression within the physiological/pathophysiological range of the target gene may be studied. We anticipate that future studies will define the pathobiological role of autocrine-paracrine systems in the local regulation of vascular tone and structure.

Another tool for testing in vitro hypotheses in vivo is transgenic/gene targeting technology. This powerful technology is extremely useful for studies of gene functions in vivo. However, transgenic technology has several disadvantages: (1) It is time consuming and costly, (2) the effect of the overexpressed transgene is exerted throughout development, (3) it is impossible to target the transgenic expression to only a local segment of a blood vessel, and (4) it is difficult to exclude the potential contribution of the systemic effect of transgene expression. If knockout of the targeted gene is lethal, it is impossible to test the specific functions by transgenic or gene-targeting techniques. In those cases, the in vivo gene transfer approach may be ideal. Thus, the local gene transfer approach may be effective for studying the role of autocrine-paracrine mediators that are complementary to transgenic technology.

### Gene Therapy in Vascular Biology

Local gene transfer technology also provides us with the opportunity to treat cardiovascular diseases. In several vascular diseases, no known effective therapy exists, eg, restenosis after angioplasty and accelerated transplant coronary vasculopathy. Because the molecular biology and pathophysiology of the vascular system is well understood, the time is ripe for the introduction of gene therapy to the management of vascular disorders. Table 3 summarizes the potential application of gene therapy for vascular diseases.

<table>
<thead>
<tr>
<th>Example of Target Gene</th>
<th>Systemic gene therapy</th>
<th>Local gene therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1. Atherosclerosis</td>
<td>1. Restenosis after angioplasty</td>
</tr>
<tr>
<td></td>
<td>2. Hypercoagulable states</td>
<td>2. Transplant rejection</td>
</tr>
<tr>
<td></td>
<td>1. Cell cycle regulatory gene</td>
<td>3. Transplant vasculopathy</td>
</tr>
<tr>
<td></td>
<td>2. Leukocyte adhesion molecule</td>
<td>4. Angiogenesis</td>
</tr>
<tr>
<td></td>
<td>3. Cytokine</td>
<td>5. Thrombosis</td>
</tr>
<tr>
<td></td>
<td>4. Fibroblast growth factor</td>
<td>6. Aortic aneurysms</td>
</tr>
<tr>
<td></td>
<td>5. Tissue plasminogen activator</td>
<td>Protease inhibitor</td>
</tr>
</tbody>
</table>

Another attractive strategy involves an antigene approach, in which targeted genes are inactivated by...
antsense oligonucleotides or plasmid. Such an approach can define the contribution of various factors to vascular pathobiology and may be used for therapy. To define the feasibility of an antigen approach for modulation of vascular structure in vivo, our initial studies have examined the determinants of VSMC growth in vivo using the rat carotid balloon-injury model. Neoinvasive growth factors are complex interactions between multiple growth factors that promote VSMC proliferation and migration, making it unlikely that selective inhibition of a particular growth factor will completely prevent lesion formation. However, growth factor–induced cell proliferation involves the sequential activation of intracellular proteins that promote cell cycle progression. Accordingly, we hypothesized that VSMC proliferation and lesion formation could be prevented by the blockade of genes regulating cell cycle progression—the final common pathway. Indeed, we have recently shown that in vivo gene transfer of antisense oligonucleotides against the cell cycle genes, proliferating cell nuclear antigen and cdc 2 kinase, markedly inhibited VSMC proliferation and lesion formation after injury in vivo.60 Similarly, Simons et al60 reported the inhibition of neointimal formation by administering antisense oligonucleotides against c-myb. We speculate that continued development of these methodologies will facilitate the use of antisense oligonucleotide technology to further characterize the biologic role of gene products activated during the pathogenesis of hypertension as well as provide potential new therapeutic agents. Although the feasibility of gene therapy for hypertension remains a remote possibility, it is clear that this experimental technology will provide new insights into the pathogenesis of hypertension.

Acknowledgments

This work is supported by grants HL-48638, HL-07708, HL-46631, HL-35252, and HL-35610 from the National Institutes of Health, Bethesda, Md, and the American Heart Association Bugeter Foundation Centers for Molecular Biology in the Cardiovascular System.

References


40. Butcher EL, Leukocyte-endothelial cell recognition: three (or more) steps to specificity and diversity. Cell. 1991;67:1033-1036.


52. Butcher EL, Leukocyte-endothelial cell recognition: three (or more) steps to specificity and diversity. Cell. 1991;67:1033-1036.


54. Butcher EL, Leukocyte-endothelial cell recognition: three (or more) steps to specificity and diversity. Cell. 1991;67:1033-1036.


81. Pratt RE, Itoh H, Gibbons GH, Dzau VJ. The role of angiotensin II in the control of vascular smooth muscle growth. 

82. Itoh H, Mukoyama M, Pratt RE, Gibbons GH, Dzau VJ. Multiple autocrine growth factors modulate smooth muscle cell 
growth response to angiotensin II. J Clin Invest. 1993;91: 
2268-2274.

83. Rakugi H, Kim DK, Krieger JE, Wang DS, Dzau VJ, Pratt RE. 
Induction of angiotensin converting enzyme in the neointima 

84. Powell JS, Glozel JP, Muller RK, Kuhn H, Hefti F, Hosang M, 
Baumgartner HR. Inhibitors of angiotensin-converting enzyme 
1989;245:186-188.

85. Kauffman RF, Bean JS, Zimmerman KM, Brown RF, Steinberg 
Ml. Inhibition by DuP 753, a non-peptide angiotensin II 
antagonist, of neointima formation following balloon injury of rat 

86. Whitebread S, Mele M, Kamber B, de Gasparo M. Preliminary 
biochemical characterization of two angiotensin II receptor 

87. Grady EF, Sechi LA, Griffin CA, Schambeian M, Kalinyak JE. 
Expression of AT2 receptor in the developing rat fetus. J Clin 

88. Takasaki I, Chobanian AV, Mamuya WS, Brecher P. Hypertension 
induces alternatively spliced forms of fibronectin in rat aorta. 

89. Sarzani R, Brecher P, Chobanian AV. Growth factor expression 
1989;83:1404-1408.

90. Roberts AB, Roche NS, Winokur TS, Burmester JK, Sporn MB. 
Role of transforming growth factor-beta in maintenance of 
function of cultured neonatal cardiac myocytes: autocrine action 

91. Shiota N, Miyazaki M, Okumishi H. Increase of angiotensin 
converting enzyme gene expression in the hypertensive aorta. 

92. Wu JN, Berceck KH. Prevention of genetic hypertension by early 
treatment of spontaneously hypertensive rats with the angiotensin 
converting enzyme inhibitor captopril. Hypertension. 1993;22: 
139-146.

93. Lee RMKW. Vascular changes at the prehypertensive phase in 
the mesenteric arteries from spontaneously hypertensive rats. 

94. Loeb AL, Mandel G, Straw JA, Bean BL. Increased aortic DNA 
synthesis precedes renal hypertension in rats: an obligatory step? 
Hypertension. 1986;8:754-761.

95. Rogg H, Schmid A, de Gasparo M. Identification and charac-
terization of angiotensin II receptor subtypes in rabbit ven-
tricular myocardium. Biochem Biophys Res Commun. 1990;173: 
416-422.

96. Wilson JM, Birinyi LK, Salomou RN, Libby P, Callow AD, 
Mulligan RC. Implication of vascular grafts lined with genetically 
mixed endothelial cells. Science. 1989;244:1344-1346.

97. Nabel EG, Plautz G, Boyce FM, Stanley JC, Nabel GJ. Recom-
binant gene expression in vivo within endothelial cells of the 

98. Dzau VJ, Morishita R, Gibbons GH. Gene therapy in the cardio-

99. Lemarchand P, Jones M, Yamada I, Crystal RG. In vivo gene 
transfer and expression in normal uninjured blood vessels using 

100. Lee SW, Trapnell BC, Rade JJ, Virmani R, Dichek DA. In vivo 
adenoviral-mediated gene transfer into balloon-injured rat 

CC, Nabel GJ. Recombinant platelet-derived growth factor B 
gene expression in porcine arteries induces intimal hyperplasia in 

CC, Maciag T, Nabel GJ. Recombinant fibroblast growth factor-1 
promotes intimal hyperplasia and angiogenesis in arteries in 

103. Kaneda Y, Iwai K, Uchida T. Increased expression of DNA 
conjugated with nuclear protein in adult rat liver. Science. 1989; 

104. Morishita R, Gibbons GH, Kaneda Y, Oghara T, Dzau VJ. In 
vivo gene transfer into intact blood vessels: a novel and efficient 

105. Dzau VJ, Gibbons GH, Cooke JP, Omoigui N. Vascular biology 
and medicine in the 1990s: scope, concepts, potential, and per-

106. Morishita R, Gibbons GH, Ellison KE, Nakajima N, Zhang L, 
Kaneda Y, Oghara T, Dzau VJ. Single intraluminal delivery of 
adenoviral-mediated gene transfer and expression in normal uninjured blood vessels using 
1993;72:1132-1138

Antisense c-myb oligonucleotides inhibit intimal arterial smooth 
V J Dzau, G H Gibbons, R Morishita and R E Pratt

Hypertension. 1994;23:1132-1140
doi: 10.1161/01.HYP.23.6.1132

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1994 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on
the World Wide Web at:
http://hyper.ahajournals.org/content/23/6_Pt_2/1132

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally
published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center,
not the Editorial Office. Once the online version of the published article for which permission is being
requested is located, click Request Permissions in the middle column of the Web page under Services.
Further information about this process is available in the Permissions and Rights Question and Answer
document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org/subscriptions/