MiniReview

This MiniReview is part of a thematic series on Cardiovascular Gene Therapy, which includes the following articles:
Prospects for Gene Therapy for Heart Failure
Gene Therapy for Restenosis
Human Gene Therapy: The Good, The Bad, and The Ugly

Charles Lowenstein, Toren Finkel, Eduardo Marbán, Guest Editors

Current Perspectives on the Use of Gene Therapy for Hypertension

Vectors for Gene Therapy
Gene Therapy for Coagulation Disorders
Cardiovascular Gene Transfer in Human Subjects

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Abstract—Systemic hypertension is a pathophysiological state that is manifested as high blood pressure and is a major risk factor for stroke, ischemic heart disease, peripheral vascular disease, and progressive renal damage. Pulmonary hypertension occurs in 3 distinct forms: primary pulmonary hypertension, pulmonary hypertension of the newborn, or secondary pulmonary hypertension attributable to a variety of lung and cardiovascular diseases. This review discusses the use of gene therapy in the control of systemic and pulmonary hypertension. Overexpression of vasodilator genes as well as antisense knockdown of vasoconstrictor genes has been successfully used in animal models of both forms of hypertension. Furthermore, the use of viral vectors to deliver these constructs has achieved long-term control of hypertension. The successful establishment of gene therapy techniques in the animal models of hypertension coupled with the anticipated advances in the genetic aspects of this disease would make it highly feasible to attempt gene delivery in the control of human hypertension. (Circ Res. 2000;87:1118-1122.)

Key Words: antisense n gene therapy n hypertension n renal and cardiac pathophysiology n renin-angiotensin system

Systemic hypertension is a complex pathophysiological state that is primarily manifested as chronic high blood pressure. If unchecked, it is a major risk factor for stroke, ischemic heart disease, peripheral vascular disease, and progressive renal damage.1-3 It is well established that a hyperactive renin-angiotensin system (RAS) plays a key role in the development and maintenance of human primary hypertension and, by some estimates, contributes to at least 10% to 30% of all cases of hypertension.4-6 It is not surprising that tremendous efforts have been directed toward the understanding of the RAS and its involvement in the control of blood pressure. The interruption of the RAS attenuates high blood pressure and many pathophysiological aspects of hypertension.1,5,7-10 Also, some genes encoding members of the RAS are associated with hypertension.11-15 Thus, it is reasonable to conclude that the RAS is essential in the normal regulation of blood pressure and pathophysiology of systemic hypertension.

Traditional agents such as diuretics, β-blockers, and calcium-channel antagonists have been used to treat systemic hypertension. Recently, a group of antihypertensive agents targeting the RAS that act by inhibiting either the formation of angiotensin II (Ang II) or the actions of Ang II have been used. These agents are reliable and affordable, and their short...
duration of action makes them an excellent choice for reversible drugs. However, they are not without limitations and disadvantages. As with most antihypertensive drugs, their effects are short-lived, have to be administered on a regular basis, and produce significant side effects. These limitations have often led to problems with compliance with some patients. Finally, all of the therapeutic agents are excellent in the control of blood pressure but hold little promise in a cure, because discontinuing the drugs results in the reappearance of high blood pressure and related cardiovascular pathological symptoms of the disease.

In addition to systemic hypertension, pulmonary hypertension is a cardiovascular disease with significant morbidity and mortality. In pulmonary hypertension, the mean pulmonary arterial pressure is >20 mm Hg at rest. This disorder occurs in 3 distinct forms: primary pulmonary hypertension, pulmonary hypertension of the newborn, and secondary pulmonary hypertension. The cellular and physiological mechanisms of this form of hypertension are poorly defined; thus, there are few effective pharmacological agents that are used clinically. The drugs presently in use are anticoagulants, calcium-channel antagonists, intravenous prostacyclin, and inhaled nitric oxide; however, none of these agents has significantly reduced the mortality attributable to pulmonary hypertension. From the discussion above it can be concluded that pharmacological regimens have reached a conceptual plateau for the treatment and long-term prevention of hypertension and that a cure for either systemic or pulmonary hypertension is not on the horizon.

For this reason, many investigators have turned their attention to explore a gene therapy strategy. They have argued that genetic manipulation may induce a permanent correction, resulting in a possible cure for hypertension. On a conceptual level, such an approach could offer major advantages over pharmacological therapy. It could essentially eliminate the compliance and side-effect issues when using conventional therapy. In addition, a genetic strategy could lead to a permanent control of hypertension if appropriate target genes controlling hypertension could be identified and their expression could be regulated.

**Gene Therapy for Systemic Hypertension**

**Overexpression of Vasodilator Genes**

Genes relevant to both vasodilation and vasoconstriction are successful targets for gene therapy in systemic hypertension. Chao and colleagues have reversed systemic hypertension in adult rats by overexpressing the vasodilator genes atrial natriuretic peptide, kallikrein, adrenomedullin, and endothelial nitric oxide synthase (eNOS). They demonstrated that delivery of these genes, either via naked DNA or viral delivery systems, in different rat models of hypertension (spontaneously hypertensive rat [SHR], Dahl salt-sensitive rat, deoxycorticosterone acetate–treated rat, and Goldblatt hypertensive rat) results in a lowering of blood pressure that is transient in nature, lasting anywhere from 6 to 12 weeks. Specifically, in the above models, the overexpression of kallikrein, atrial natriuretic peptide, adrenomedullin, and eNOS caused a 22 to 50, 22, 28, and 21 mm Hg change in blood pressure, respectively, over a period of 6 to 12 weeks after delivery. This decrease in blood pressure is accompanied by a transient attenuation of some pathophysiological changes observed in the major target organs (heart, kidney, and blood vessels) associated with hypertension.

**Antisense Knockdown of the RAS**

The use of antisense gene therapy to target vasoconstrictor pathways has been quite successful in lowering blood pressure and preventing or reversing the associated cardiovascular pathophysiology of hypertension on a long-term basis. The RAS has been the target of choice for antisense gene therapy in the treatment of systemic hypertension. There are multiple reasons for this. First, the role of the RAS in hypertension is well understood. Second, the RAS provides an ideal target for gene delivery, because it is widely distributed. Third, pharmacological agents that target the RAS exert antihypertensive effects at multiple levels (vasodilator mechanisms and fibrinolytic and oxidative stress pathways) in addition to their actions on Ang II.

Conceptual support that antisense targeting of the RAS would be effective in treating hypertension was derived from the use of antisense oligonucleotides. Studies demonstrated that the central or peripheral injection of antisense oligonucleotides to the RAS (angiotensinogen or angiotensin II type 1 receptor [AT1 R]) resulted in a transient, short-term (days), and modest, but significant, effect on lowering blood pressure in the SHR. The amount (~23 mm Hg) and duration (9 weeks) of the blood pressure-lowering effect were prolonged to weeks with the use of an adeno-associated virus vector delivery system.

Our research group has used these studies as a basis to determine if retroviral-vector–mediated delivery of antisense targeting the AT1 R and angiotensin-converting enzyme (ACE) would prevent hypertension (high blood pressure) and cardiovascular pathophysiology associated with hypertension for longer time periods. A single intracardiac administration of retroviral particles containing AT1 R antisense (AT1 R-AS) into 5-day-old SHR results in attenuation of high blood pressure and long-term expression of the AT1 R-AS for at least 210 days. This attenuation of blood pressure is similar to that seen for AT1 R antagonists. This was associated with complete attenuation of cardiac hypertrophy, arterial wall thickness, and perivascular and myocardial fibrosis.

AT1 R-AS expression also resulted in significant reduction in the amount of neointimal formation after carotid artery balloon injury in the SHR (Figure). AT1 R-AS prevented alterations in vascular reactivity, endothelial dysfunction, Ca²⁺ handling, and ion channel dysfunction in renal arterioles. These studies established that AT1 R-AS will abolish the development of hypertension in the SHR on a long-term basis.

Intracardiac injection of virus particles containing AT1 R-AS into the adult SHR resulted in a 30- to 60-mm Hg reduction in blood pressure that was maintained for up to 36 days compared with the SHR treated with virus alone. This was accompanied by a reversal of the increase in vasoreactivity and the gain of endothelial function in renal resistance arterioles. These observations are consistent with studies that
have previously shown a similar transient reversal of high blood pressure by antisense in the adult rat. Collectively, these data demonstrate that virally mediated gene delivery of AT1R-AS can effectively reduce blood pressure and reverse renovascular pathophysiology associated with hypertension in the adult SHR. However, the transient nature of the antihypertensive effect in the adult remains to be addressed and must be improved on.

Also relevant would be determining if antisense gene targeting could clarify the role of the tissue RAS in cardiovascular pathophysiology. This is clinically relevant in view of the recent results from the Heart Outcomes Prevention Evaluation clinical study. This study showed that ramipril, an ACE inhibitor, produced a significant improvement in outcomes and reduction in death attributable to cardiovascular causes without a significant change in blood pressure. Introduction of ACE antisense (ACE-AS) by a retroviral vector resulted in a modest (15 to 18 mm Hg) but significant attenuation of high blood pressure exclusively in the SHR for 100 days. In spite of this modest decrease in blood pressure, ACE-AS resulted in complete attenuation of venous pressure, decreased cardiac hypertrophy and fibrosis, decreased medial thickness, and normalization of renal artery excitation-contraction coupling, Ca2+ current, membrane potential, K+ current, and [Ca2+]i compared with offspring derived from the virus-treated SHRs. In addition, AT1R-AS was found in the genomic DNA and was expressed in cardiowascularly relevant tissue of the F1 and F2 offspring. The transmission of antihypertensive phenotypes to the offspring was also seen in the ACE-AS–treated SHRs. The mechanism of transmission of this antihypertensive phenotype to the offspring is unknown at the present time but might involve germ line transmission of the antisense, parental environment, transmission of a humoral factor through the breast milk, or expression at a critical stage of SHR development. For example, cross studies have shown that SHR babies parented by WKY mothers express a significantly lower blood pressure, while WKY babies parented by SHR mothers are hypertensive.

Antisense Knockdown of the β1-Adrenergic Receptors

Zhang et al have used antisense oligonucleotides to the β1 receptor to provide additional conceptual support for gene therapy in hypertension. A single intravenous injection of β1 receptor antisense in cationic liposomes decreased cardiac β1 receptor density by 30% to 50%, attenuated the β1 receptor–mediated positive inotropic response in isolated perfused hearts, and decreased blood pressure by ≈38 mm Hg in the SHR. The effect lasted 2 to 3 weeks and, interestingly, had no significant effect on heart rate. In addition, a 35% reduction in the renal β1 but not β2 receptor density was observed. Finally, 4 days after administration, β1 receptor antisense decreased preprorenin mRNA levels in the renal cortex by 37%, which was accompanied by a marked diminution of plasma renin activity and plasma Ang II levels by day 10.

Gene Therapy for Pulmonary Hypertension

It has only been in the last 2 years that gene therapy for treatment of pulmonary hypertension has begun. The overexpression of vasodilator genes, eNOS, prepro-calcitonin gene–related peptide (CGRP), and prostaglandin I synthase (PGIS) has shown great promise in the laboratory for treatment of pulmonary hypertension. Patients with severe pulmonary hypertension have a PGIS deficiency of their precapillary vessels, but the importance of this deficiency for lung vascular remodeling remains unclear. Geraci et al hypothesized that selective pulmonary overexpression of PGIS may prevent the development of pulmonary hypertension. Transgenic mice were created with selective pulmonary PGIS overexpression. The mice overexpressing PGIS produced 2-fold more pulmonary 6-keto prostaglandin F1α than controls, and, after exposure to chronic hypobaric hypoxia, the PGIS-overexpressing mice showed a lower right ventricular systolic pressure than controls. Histological examination of the lungs revealed nearly normal arteriolar vessels in the PGIS-expressing mice in comparison with vessel-wall hypertrophy in the control mice. These studies demonstrate that overexpression of PGIS in the lung protected mice from the development of pulmonary hypertension after exposure to chronic hypoxia.

Using adenoviral gene transfer of eNOS to the mouse lung, Champion et al showed the beneficial effects of this gene on...
pulmonary hemodynamics. At 21 to 28 days after gene transfer, the pressure-flow relationship in the pulmonary vascular bed was shifted to the right in animals transfected with eNOS, and pulmonary pressor responses to endothelin-1, Ang II, and ventilatory hypoxia were reduced significantly in animals transfected with the eNOS gene. In preliminary findings, this same group demonstrated that not only did the eNOS knockout mouse have pulmonary hypertension (pulmonary artery pressure ≈25 mm Hg), but when the eNOS gene was delivered back to the lung of the knockout mouse using an adenoviral construct, no pulmonary hypertension developed in the knockout.43 CGRP is also believed to play an important role in maintaining low pulmonary vascular resistance and modulating pulmonary vascular responses to chronic hypoxia. Intratracheal administration of prepro-CGRP with an adenovirus to the mouse lung, followed by 16 days of chronic hypoxia, caused an attenuation of the hypoxic-induced increases in pulmonary vascular resistance, right ventricular mass, pulmonary artery pressure, and pulmonary vascular remodeling.45 These recent findings suggest that the use of gene therapy targeting vasodilator agents in the lung may be successful in the treatment of pulmonary hypertension.

**Future Directions and Conclusions**

It is evident from the above discussion that the use of gene therapy is an exciting approach that is intellectually sound and, on the basis of animal experiments, may hold great promise for a permanent treatment of hypertension. However, before leaping to the next step (eg, clinical trials), several important issues must be resolved and hurdles must be cleared. First, are the viral vectors the most effective means to deliver an antihypertensive gene? Benefit versus risk consideration of viral vectors may lead us to compromise and develop better means to deliver more effective and highly stable naked DNA that will have prolonged duration of action of desired effects. However, if viral vectors are the method of choice, they must meet strict safety requirements. For example, it would be imperative that the integrations site of the viral vector in the genome be known and that its influence on neighboring genes be evaluated and proven to be free of immune and other adverse side effects.

Second, other gene targets that may be relevant in hypertension must be explored. For example, the targeting of matrix proteins could be a potentially interesting site of gene therapy for hypertension. This is relevant in view of the fact that therapies using secreted molecules may also be beneficial. Therefore, it seems plausible that targeting extracellular matrix proteins may also be beneficial in vascular disorders. It would also be interesting to develop a strategy that would target antisense knockdown of Ca2+ channels using a smooth muscle cell–specific promoter. Finally, cell-specific overexpression of signaling molecules that may be vasodilator in nature (ie, protein kinases A and G) would be potential targets for antihypertensive gene therapy.

In conclusion, there is sufficient evidence to indicate that gene therapy may be an important and significant step forward in the long-term treatment and possible cure of both systemic and pulmonary hypertension. It is quite possible that hypertension-relevant genes could be identified in the near future. The establishment of the conceptual basis for gene therapy in the animal models of hypertension coupled with the anticipated advances in the genetic aspects of this disease would make it highly feasible to attempt gene delivery in the control of human hypertension.

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