The Future of Hypertension Therapy: Sense, Antisense, or Nonsense?

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Abstract—Hypertension is a debilitating disease with significant socioeconomic and emotional impact. Despite recent success in the development of traditional pharmacotherapy for the management of hypertension, the incidence of this disease is on the rise and has reached epidemic proportions by all estimates. This has led many to conclude that traditional pharmacotherapy has reached an intellectual plateau, and novel approaches for the treatment and control of hypertension must be explored. We have begun to investigate the possibility of treating and/or curing hypertension by using genetic means. In this review, we will provide evidence in favor of targeting of the renin-angiotensin system by antisense gene therapy as an effective strategy for the lifelong prevention of hypertension in the spontaneously hypertensive rat model. In addition, we will discuss the properties of an ideal vector for the systemic delivery of genes and the potential experimental hurdles that must be overcome to take this innovative approach to the next level of evaluation. (Hypertension. 2001;37[part 2]:357-364.)

Key Words: renin-angiotensin system n genes n rats, spontaneously hypertensive n hypertension, genetic n antisense elements

Hypertension is a debilitating disease that affects approximately 50 million Americans. It significantly increases risks for such disorders as coronary artery disease, stroke, cardiac arrhythmia, heart failure, cardiomyopathy, valvular heart disease, abnormal renal function leading to renal failure, and many other complications associated with structural damage to the cardiovascular system. The underlying cause of hypertension is poorly understood. Only a small percentage of hypertensive patients express demonstrated genetic abnormalities. These include mutations in the 11–β-hydroxy steroid dehydrogenase gene and mutations in certain renal ion channels such as those found in Liddle’s and Gordon’s syndromes. However, in the majority of patients, the cause of hypertension is unknown and falls into the category of “primary hypertension.” This has led many investigators to hypothesize that expression of the hypertensive phenotype is multifactorial and may involve the interactions and variations of multiple genes.

Two classes of genes have attracted particular attention not only in the elucidation of the cellular and physiological mechanisms of hypertension but also in the development of traditional pharmacological therapies for the disease. They include vasodilatory genes such as bradykinin, nitric oxide synthase, and atrial natriuretic peptide (ANP) and vasoconstrictor genes such as the renin-angiotensin system (RAS) components, endothelin, adrenergic receptors, and calcium channels. In fact, major strides have been made in the development of drugs targeting these two classes of genes and have resulted in fairly successful management and control of hypertension. The principal reason for the success of traditional pharmacological agents in the treatment of hypertension has been their reliability, affordability, and reversibility of action. As a result, they are excellent for the management of both short-term and long-term disease. However, several drawbacks are associated with traditional therapy. First, end-organ damage, a hallmark of the hypertensive state, may have already occurred once hypertension is diagnosed and therapy initiated. As a result, many of the current therapeutic agents sometimes are unable to reverse the end-organ damage and other pathophysiological complications associated with hypertension. Second, patient compliance is a critical issue because of the side effects induced by some drugs and because mild to moderate hypertension is usually asymptomatic, patients often view pharmacotherapy as unnecessary and inconvenient. Finally, the conventional pharmacological strategy is successful in the control and management of hypertension, but it does not provide a long-term control for the disorder. As a result, the disease generally is reexpressed once therapy is discontinued. These observations have led many investigators to suggest that traditional pharmacological therapy has reached an intellectual plateau. This view is further supported by the fact that the
incidence of hypertension and cardiovascular diseases has been steadily rising and in fact has reached epidemic proportions. Thus, we believe that new and innovative approaches must be discovered to control and cure this disease. Our objective in this review is to develop arguments and present evidence that a novel approach could be antisense gene therapy. In an attempt to do so, we will present the rationale for using the RAS as a target for gene therapy, discuss various vectors to deliver the therapeutic genes, and will provide “proof of principle” in support for the anti–RAS-based gene therapy. Finally, we will discuss major hurdles that must be overcome to move from the experimental stage to its consideration for use in human hypertension.

**Sense Versus Antisense: Why Target the RAS?**

Gene therapy provides a powerful tool to influence the expression of a specific gene to compensate for the hypoactivity or hyperactivity of a defective gene. This can be achieved by overexpression of a normal gene or by suppression of a defective gene. In fact, both approaches have been used for the control of hypertension. For example, Chao and collaborators have been very successful in reversing hypertension in adult rats by overexpressing the vasodilatory genes: ANP, kallikrein, adrenomedulin, and endothelial nitric oxide synthase. Their studies have demonstrated that delivery of each gene, either by naked DNA or by using viral delivery, results in an impressive lowering of blood pressure (BP) that was transient in nature, lasting anywhere from 6 to 12 weeks in different models of hypertension. This decrease in BP was accompanied by a transient attenuation of some pathophysiology observed in the major target organs (heart, kidney, and blood vessels) associated with hypertension. For example, adeno-virus-mediated kallikrein gene delivery to DOCA salt-hypertensive rats caused a significant reduction in urinary protein excretion, glomerular sclerotic lesions, brush border disruption of proximal tubules, tubular dilation, and protein cast accumulation. Similarly, human endothelial nitric oxide synthase plasmid DNA delivery to the spontaneously hypertensive rat (SHR) induced significant increases in urinary and aortic cGMP and urinary and serum nitrite/nitrate, without a significant effect on body weight, heart rate, water intake, or food consumption. These groundbreaking studies provided conceptual support for the usefulness of the “sense” approach for the control of hypertension.

In contrast, the “antisense” approach has been developed to target the major vasoconstrictor pathways. The basic principle of the antisense approach is that it blocks the formation of the targeted protein rather specifically either at the transcriptional or translational level. This is achieved by the formation of RNA-RNA hybrid complexes between the antisense and endogenous RNA molecules. Other mechanisms of antisense actions include its binding to cellular proteins, and the use of ribonuclease H to cleave RNA-DNA hertoduplexes are other possibilities. Our research group has chosen to target the RAS with this antisense approach to provide conceptual support for its usefulness in hypertension. There are multiple reasons for this choice: (1) the role of the RAS in hypertension is well understood, (2) the RAS provides an ideal target for gene delivery because it is widely distributed, (3) traditional pharmacological agents that target the RAS are proven potent antihypertensive medications. As a result, there are well-developed protocols that can be used to compare the outcomes of antisense gene therapy with traditional pharmacological inhibitors.

The first conceptual support that antisense targeting of the RAS would be effective for the treatment of hypertension was derived from the use of antisense oligonucleotides. Some investigators demonstrated that the central or peripheral injection of antisense oligonucleotides to RAS components (angiotensinogen or the angiotensin [Ang] II type I receptor) results in a significant lowering of BP in the SHR. This effect persisted for days. The duration of this BP-lowering effect was prolonged to weeks with the use of viral vector-mediated delivery of antisense. Our research group has built on these observations and has used a retroviral vector gene delivery system to produce lifelong transduction of the AT1 R-antisense (AT1,R-AS) resulting in permanent control of high BP and both vascular and cardiac pathophysologies associated with hypertension. It is evident from the above discussion that both “sense” and “antisense” strategies are technically sound and exciting approaches that offer innovative means for the long-term control of hypertension. However, we believe that the antisense strategy may offer advantages over the sense approach: (1) The efficacy and efficiency of the antisense strategy can be easily compared with the traditional pharmacological approach. Such a comparison is difficult for the sense-based strategy because of the lack of an equivalent pharmacological parallel. (2) Introduction of a full-length gene that would result in a physiologically functional compensation would require a higher degree of transduction in vivo. Nonetheless, both approaches must be developed to further evaluate their potential as antihypertensive therapy. However, we also believe that the key to their success will be dependent on the availability of delivery vehicles that could deliver a desired gene to specific organs with high efficiency and specificity to maintain long-term expression of the transgene, which could be regulated on demand. Development of such an ideal vector would be an important next step.

**Gene Delivery Vehicles**

Currently, several methods are in use to facilitate the entry of nucleic acids into cells, each of them with their own advantages and disadvantages. They can be divided into nonviral and viral vector–mediated systems. There is a wide variety of nonviral vector systems that have been used to deliver genes in vivo for the control of hypertension with some success. Although this method is safe, it does not appear to have great promise for long-term or permanent control of the disease. Liposomes are the most often used method for the delivery of genes by nonviral means. They are nonpathogenic, easily produced, and do not have a size constraint. However, the inability of liposomal-delivered DNA to integrate into the recipient cell genome makes them less suitable as long-term antihypertensive agents.

Viral vectors, on the other hand, have become increasingly popular gene delivery vehicles. They are replication-defective viral particles that retain the ability to enter the target cells
and transfer (transduce) their genetic material. Replacing genes required for viral replication with an expression cassette containing the therapeutic gene(s) transforms the viruses into safe vectors. Several viruses have been developed as possible vectors, each one of them exhibiting unique qualities. These include retroviruses and adeno-associated viruses (AAV), herpes simplex viruses, and the lentivirus-based HIV-1 vector. Retrovirus-based vectors integrate into the DNA of the host cell, providing the potential for long-term transgene expression. They are highly efficient at infecting dividing cells and thus are the vector of choice for cancer therapy. However, new generations of retroviral vectors that are less immunogenic, have the improved ability to infect nondividing cells, and can integrate into specific locations in the host genome would be ideal for hypertension therapy. In contrast to retroviral vectors, adeno-associated viruses infect nondividing cells with high efficiency. However, their potential to induce immunogenic responses and their episomal localization makes them less ideal for hypertension therapy. Recently, there have been efforts to develop a chimeric viral vector, which exhibits the retroviral properties of integration and low immunogenicity and adenoviral properties of the ability to infect nondividing cells. Such a vector would be ideal for the advancement of this field and should be tested for its in vivo use in hypertensive animal models. HIV-based vectors, which also fall under the category of the retroviral vector, are a recent addition with great promise. They combine the advantages of retroviral and adeno-associated vectors and may turn out to be the vectors of the future. This is because they have high efficiency of transduction, infect nondividing cells with the same efficiency as dividing cells, and have large genomes to introduce multiple transgenes. Finally, AAV vectors have garnered significant interest as well because of their safety and ability to infect nondividing cells. However, because of their limited genome capacity (≈4 kb), their use for hypertension research is limited at the present time.

What would be an ideal vector for systemic gene transfer for hypertension research? That would be a vector that can transduce nondividing and dividing cells with equal efficiency, is easily produced on a large scale, expresses minimal or no immune and other adverse effects, has a high capacity in its genome to introduce regulatory elements, and can integrate in the host genome without influencing other genes. No vector is currently available that satisfies all of these requirements. However, this field is rapidly developing and probably would lead to the discovery of such a vector in the near future.

Prevention of Hypertension by Antisense Gene Therapy: “Proof of Principle”

We set out to determine if long-term expression of antisense gene against the AT receptor subtype \( AT_R \) by a retroviral vector-mediated (LNSV) delivery system in vivo would be successful in the SHR. The rationale for choosing the \( AT_R \) as a target were several: (1) \( AT_R \) antagonism is a proven traditional pharmacological strategy for the control of hypertension, (2) a polymorphic substitution of adenine with cytosine in the \( AT_R \) gene has been associated with severe hypertension, (3) interactions between ACE and \( AT_R \) activity has been associated with the hypertensive state.

Intracardiac administration of a retroviral vector containing the \( AT_R-AS \) gene in 5-day-old SHR produces long-term antihypertensive effects in the adults. The attenuation of high BP persisted throughout life, is comparable with that of other \( AT_R \)-antagonists, and is exclusive to the SHR because no effect on basal BP is observed in normotensive rats. The delivery of the \( AT_R-AS \) gene is also associated with the attenuation of cardiac pathophysiology. This includes the prevention of cardiac hypertrophy, perivascular and myocardial fibrosis, and inhibition of neointimal hypertrophy/hyperplasia in the coronary arterioles. In addition, significant attenuation of neointimal formation after carotid artery balloon injury was observed in SHR. Examination of the renal resistance arterioles showed significant changes after \( AT_R-AS \) treatment. Alterations in vascular reactivity, endothelial dysfunction, \( Ca^{2+} \) handling, and ion channel dysfunction in the renal arterioles were prevented. The prevention of hypertension for life in the SHR is associated with the robust and long-term expression of the \( AT_R-AS \) gene in cardiovascular-relevant tissues such as the adrenals, heart, kidney, and vessels. Finally, the expression of the \( AT_R-AS \) gene is associated with a 20% to 40% decrease in the numbers of \( AT_1 \) receptors in these tissues. It is relevant to point out that such a modest decrease in the receptors may be directly linked to long-term effects. Alternatively, other mechanisms leading to a decrease in the RAS activity indirectly cannot be ruled out at the present time. These observations indicate that a single intracardiac injection of a retroviral vector containing \( AT_R-AS \) prevents hypertension in the SHR for life without any visible side effects. In contrast to traditional \( AT_R \) antagonist therapy, \( AT_R-AS \) treatment does not increase plasma Ang II levels.

These exciting studies provided evidence for the “proof-of-principle” that antisense gene therapy may be a feasible approach for the treatment of hypertension. However, they also raised many questions: (1) Can inhibition of ACE by ACE-AS produce a similar antihypertensive effect? The answer is most definitely yes. We have established that a single intracardiac injection of a retroviral vector containing ACE-AS causes modest but significant decreases in high BP as seen for the \( AT_R-AS \). (2) Are the antihypertensive effects of the \( AT_R-AS \)-antisense therapy specific? Our studies demonstrate that \( AT_R-AS \) expression has no significant effect on the expression of \( AT_1 \) receptors and that phenylephrine-induced vascular responses are not altered in the \( AT_R-AS \)-treated rats. These data argue in favor of \( AT_R-AS \) specificity. (3) What is the cellular localization of the \( AT_R-AS \)? Systemic delivery of the viral vector has been chosen to mimic the traditional pharmacological strategy, in which all the cardiovascular tissues have access to the drug. This route of administration results in an integration of the \( AT_R-AS \) and its expression in various tissues. Further, tissue distribution of the vector was carried out with the use of an enhanced green fluorescent protein (EGFP)-expressing retroviral vector. A high degree of EGFP transduction was evident in the hepatocytes (Figure 1A) and in the endothelial...
cells of the aorta (Figure 1B). In addition, limited numbers of vascular smooth muscle cells (VSMC) appear to be transduced by the LNSV-EGFP (Figure 1B). This indicates that the retroviral vector, when delivered in a neonatal rat, can transduce endothelial and VSMC, 2 cell types important for the control of vasoconstriction. (4) Is the expression of the $AT_{1R}$-AS of any consequence in the normotensive rat? Our studies have established that although $AT_{1R}$-AS/ACE-AS is equally expressed in both normotensive rats and SHR, the expression is of little consequence on basal BP in normal rats. This is despite the fact that the $AT_{1R}$ numbers and Ang II–induced dipsogenic and BP responses are modestly attenuated. These observations are consistent with the traditional pharmacological strategy in which both $AT_{1R}$ antagonists and ACE inhibitors express little or no effect in normal individuals. It further supports a long-held view that the RAS is of little relevance in the control of normal BP as the result of the existence of many other interacting physiological mechanisms. These views led us to propose that the expression of $AT_{1R}$-AS in the normal rat would only come into play when the RAS is challenged. In fact, our recent observations support this hypothesis. $AT_{1R}$-AS–expressing normotensive rats, when challenged with chronic low-dose Ang II (55 ng/kg per minute), were completely protected from developing hypertension.

Future Perspectives

Our studies have established that genetic targeting of the RAS prevents hypertension for life in the SHR. These observations are consistent with studies from other groups. Thus, they provide evidence that this strategy is technically feasible and innovative and could be the basis for the treatment and possible cure of human hypertension. However, major hurdles must be overcome, better vectors must be developed, and the mechanisms of antisense action must be elucidated before such a leap ever becomes a reality. Some of these issues are discussed below:

Test of Antisense Strategy With Other Animal Models of Hypertension

For the antisense gene therapy to be useful, we must prove that it is effective in many other forms of Ang II–dependent hypertension and test its usefulness in other models. The
renin-transgenic rat, a monogenetic model of hypertension based on the overactivity of the tissue RAS, has been used to provide further evidence in support of this concept. These studies demonstrate that a single intracardiac administration of AT,R-AS completely attenuates cardiac and vascular pathophysiology with a modest but significant decrease in high BP (unpublished data). A fructose diet induces both high BP and insulin resistance. This was completely prevented in rats harboring AT,R-AS. These data are encouraging; however, other animal models (ie, DOCA salt, Dahl salt-sensitive rat, Goldblatt hypertensive rat) must be investigated to further establish the efficacy of this therapeutic intervention.

Reversal of Hypertension

Thus far, our efforts have focused on the preventive aspects of hypertension. Because there are no reliable genetic markers for hypertension, this approach is not applicable for the treatment of human hypertension. We must determine if antisense gene therapy has the potential to reverse established hypertension on a long-term basis. Initial studies from our group and those of others provide encouraging results. Intracardiac administration of retroviral or AAV vector containing AT,R-AS into adult SHR or double-transgenic mice results in a significant decrease in high BP that was maintained for weeks.9,10,44 This was accompanied with reversal of the increased vasoreactivity and gain of endothelial function in renal resistance arterioles. Chao and associates45,46 have used the "sense" approach with the kallikrein and ANP genes to reverse hypertension transiently through the use of adenoviral vectors. These are important accomplishments indicating that the strategy is feasible in reversing hypertension. However, the transient nature of the antihypertensive effect must be addressed, and approaches must be developed with the use of better viral vectors to prolong this effect.

In the last several years, HIV vectors have proven to be highly efficient in transducing a wide variety of nondividing cells and could be an ideal vector to test. They are nonimmunogenic, have a capacity of ~10 kb for genetic material, and sufficient amounts of vector at high concentrations (10^8 to 10^9 infection units/mL) can easily be produced. Despite these advantages, safety has been a major concern with the use of HIV-derived vectors. However, over the years, many modifications have been made to reduce the risk associated with the use of this gene delivery system. The necessary components for the production of the recombinant virus are segregated on 3 different plasmid constructs (Figure 2). Homologous sequences between these constructs have been reduced to the bare minimum to combat recombination. The "helper" plasmid provides the necessary HIV-1 proteins in trans expressed from the human cytomegalovirus immediate early promoter (CMV IE ), with some additional key deletions in the construct.47,48 The second plasmid is an envelope-encoding plasmid, usually the glycoprotein from the vesicular stomatitis virus (VSV). This "pseudotype" envelope provides superior stability and the ability to ultraconcentrate the vector. The third plasmid is the "transducing" plasmid. This contains a functional packaging signal, a transgene expression cassette, and viral long-terminal repeats (LTRs) containing self-inactivating (SIN) mutations. The SIN mutation silences the promoter activity of the upstream LTR and decreases the risk of replication-competent virus generation. As a result of these modifications, the HIV-1 has become a safe, efficient, and well-understood vector system.

HIV-lacZ and HIV-GFP were constructed to determine the transduction efficiency of these vectors in vitro and in vivo in nondividing cells. Infections of primary neurons at 10 MOI
Systolic BP (mmHg)

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**Figure 4.** Tet-regulatable expression of AT₇R-AS in SHR. Five-day-old SHRs were treated with both vectors simultaneously (1 × 10⁷ cfu/mL, transactivator + AT₇R-AS) as described previously. Animals (6 in each group) were allowed to grow to adulthood and on day 60 were administered dox in drinking water. Analysis of AT₇R-AS transcript and measurement of systolic BP were carried out (P<0.05, no dox versus dox).

(multiplicity of infection) showed >90% transduction efficiency (Figure 3A). A single intracardiac injection of HIV-lacZ (1 × 10⁷ IU/mL) demonstrated a successful transduction of cardiac cells (Figure 3B). In addition, endothelial cells of the aorta were also transduced (Figure 3C). It is pertinent to point out that the concentrations of HIV vector used for this study was ~100-fold lower than the LNSV doses, confirming a profound increase in the transduction efficiency with this new vector. These data have ideally poised us to test the efficacy of the HIV-AT₇R-AS encoding virus in adult, hypertensive animals.

**Constitutive Versus Regulated Gene Delivery Systems**

The expression of the antihypertensive transgene must be regulated by exogenous means. This is crucial not only to control the degree of transgene expression and thus therapeutic response but also to turn off its expression as a result of any unforeseen side effects. In addition, it is difficult to study the reversible effects with constitutive systems, which are an integral part of “acquired” diseases such as atherosclerosis, restenosis, and hypertension in which environmental and polygenic factors play an important role. A number of regulated gene expression systems that use exogenous ligands to control transgene expression have been developed in recent years. These systems use exogenous ligands such as mifepristone, rapamycin, and tetracycline to act on transactivators containing natural or mutated ligand binding domains.⁹⁰⁻⁹² This facilitates specific and regulated transcription of a transgene. One such system that has gained widespread attention is the tetracycline (tet) inducible system, which consists of a tet transactivator and tet operator sequence.⁹⁰,⁹⁹ This 2-vector system, when administered to the host, synthesizes the tet transactivator protein. Administration of tetracycline results in binding of the drug to the transactivator protein, which initiates transcription of the desired transgene. Withdrawal of tet results in the cessation of transgene transcription, thus facilitating specific and regulated transcription of a therapeutic gene of choice.

Preliminary experiments have been carried out to determine if such a system works in the SHR. The tet transactivator and AT₇R-AS genes were cloned into separate vectors. Viral particles (1 × 10⁹ cfu/mL) containing tet transactivator and AT₇R-AS were injected simultaneously in 5-day-old SHRs. Animals were given doxycycline (dox) in their drinking water on day 60 to turn on the expression of AT₇R-AS. Control rats did not receive dox. Twenty-one days after initiation of dox treatment, AT₇R-AS transcript expression and mean BP were measured. Figure 4 shows that dox was able to turn on the expression of AT₇R-AS. This was associated with a significant reduction in high BP. These data, although preliminary, indicate that tet inducible system is tightly regulated in the SHR and can be studied further for its usefulness in regulated expression of antihypertensive responses. We must also investigate the tet off system to circumvent the compliance issue related to the use of “tet” on a regular basis.

**Safety and Route of Delivery of Viral Vectors With Therapeutic Genes**

Safety of viral vectors should be of great concern if it is to be the therapeutic method of choice. It is imperative that the site of integration of the viral vector in the host genome be known, and its influence on neighboring genes must be established. In addition, the vector must undergo a proven safety analysis and be free of immune and other adverse side effects. Because retroviral vectors and the AAV are integrated into the DNA, we must establish if there is any germ line transmission of these transgenes. Preliminary data from our retroviral vector experiments raise this concern.³⁴ Physiological, pathophysiological, and ethical aspects related to germ line transmission of the vector must be discussed, and its implication must be evaluated by weighing the risks and benefits from the treatment of this disease.

The route of administration of the viral vector must be improved. Initial studies have used systemic delivery in which the vector is immediately available for infection to all the tissues. It is not the most efficient delivery route because the hepatic and pulmonary systems would extract most of the vector before it reaches the cardiovascular-relevant tissues. Tissue-specific targeting with the use of either direct injection of the vector (ie, muscle, cardiac, subcutaneous) or the use of tissue specific promoters (ie, vascular smooth muscle, endothelial-specific) must be attempted.

**Other Genes as Targets for Hypertension Gene Therapy**

Although antisense gene therapy targeting the RAS has provided encouraging results, other hypertension-related
genes must be tried in an attempt to improve the therapeutic outcome. For example, Ca²⁺ channels in the vasculature, signaling molecule-related genes (i.e., protein kinase A/G or SERCA) and genes relevant to matrix proteins, all offer interesting possibilities as potential sites for hypertension gene therapy.

Conclusions
All the existing data indicate that gene therapy based on either the overexpression of vasodilatory genes or the inhibition of vasoconstrictor genes is an exciting pharmacotherapeutic approach, which holds great potential for the treatment of hypertension in the future. It appears that this gene therapy approach is conceptually sound and may provide a means for the permanent control and possible cure of hypertension. However, we must use extreme caution and restraint. Extensive experiments must be carried out to establish the mechanisms, and new and safe viral vectors that have high transduction efficiency must be developed. An in vivo-regulated system must be tested. Each of these components must be completed before we can attempt to try the gene therapy approach in human hypertension. We, as physiologists, are in an excellent position to develop collaborations with pharmacologists, molecular biologists, virologists, and geneticists in an attempt to reach this goal.

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