Potential of Gene Therapy Strategy for the Treatment of Hypertension

Mohan K. Raizada, Shant Der Sarkissian

Hypertension is one of the most important risk factors for cardiovascular diseases (CVDs). CVD extends across all populations and represents the leading cause of morbidity and mortality in industrialized countries. Hypertension increases the risk of peripheral arterial disease, cardiomyopathy, stroke, and renal failure. The disease affects ≈50 million Americans and goes undetected in at least one third of the population. In spite of the tremendous progress made by pharmacotherapy in the management and control of hypertension, there has been a steady escalation in its prevalence during the last decade. This has led many to conclude that traditional pharmacotherapy has reached an intellectual plateau and that novel and innovative approaches must be sought for the treatment, control, and possible cure of hypertension. As a result, efforts of our group and those of many others have been diverted to explore the use of gene transfer and gene therapy strategies for a long-term control of hypertension. We have argued that gene therapy offers potentially significant improvements and benefits over the use of traditional pharmacotherapy: (1) noncompliance by patients can be significantly reduced or even eliminated because of the fact that a single treatment involving gene transfer could remain effective for months or even years; (2) side effects associated with pharmacotherapy can be minimized as a result of specific targeting of a therapeutic gene in a given tissue and optimally influencing cardiovascular pathophysiology on an individual patient basis; and (3) with its potential to produce long-term beneficial outcomes in end-organ damage, the gene therapy strategy could lead to a cure of hypertension and its related pathophysiology. It is noteworthy to mention that current pharmacotherapies are often unable to reverse end-organ damage associated with hypertension.

In order for the gene therapy for hypertension to be successful, one must provide conceptual support of its efficacy in animal experiments. This includes identification of effective target gene(s) that are linked to the hypertensive state, development of an ideal viral vector system that would be highly effective in the transduction of a transgene into cardiovascular relevant tissues, and identification of an appropriate regulatable promoter that would enable a controlled expression of a hypertension-relevant gene. Our objectives in this review are to address these issues and to present our views as to the future of gene therapy to move this strategy from animal experimentation to clinical trial levels.

Ideal Vector for Hypertension Gene Therapy

An ideal vector for gene transfers for the control, reversal, and, possibly, cures for hypertension must possess the following properties. First, the vector should be able to transduce nondividing and slowly dividing cells with high efficiency. This is a critical requirement, because most of the cardiovascular-relevant cells are already quiescent and terminally differentiated by the time the disease is fully expressed. Second, the vector should have the ability to integrate into the host genome for a long-term and persistent expression of a therapeutic gene. Third, the vector must possess sufficient capacity for the introduction of regulators and promoters. Fourth, the vector should be easily produced in large scale and in highly pure form. Lastly, the vector should be safe, and its integration site in the host genome must be such that it does not cause disruption or mutation of other genes.

Currently, no such perfect vector exists, although major strides have been made toward this goal, and the availability of an ideal vector is within our reach. Many excellent vectors, including the adeno-associated virus (AAV) and lentiviral vectors, are currently in use to carry out preclinical experiments and to provide “proof of concept” for gene therapy hypertension and CVDs. Each of these vectors possesses its advantages and disadvantages as reviewed recently.1

Gene Therapy Strategies

Over the years, 2 contrasting strategies have emerged for gene therapy in hypertension: the “knockdown” or gene expression reduction approach and the “overexpression” or gene induction approach.1-3 In the knockdown approach, genes that have been implicated in the elevation of BP and cardiovascular pathophysiology (particularly members of the renin–angiotensin system [RAS]) are targeted with the anticipation of decreasing their transcription and translation. With the overexpression approach, genes that are relevant in reducing high blood pressure (BP), such as kallikrein, atrial natriuretic peptide (ANP), endothelial nitric oxide synthase (eNOS), and heme oxygenase, are overexpressed.4 Finally, transgenic approaches to alter the genetic makeup of an...
animal have been successful in reducing high BP or preventing the rise in BP in experimental animals. However, a potential pitfall in this approach is that animals may undergo altered physiology to compensate for transgene expression. This would be particularly critical if the concerned transgene plays a major role in the developmental process. Thus, we believe that experimental genetic manipulations for gene therapy should be conducted after the developmental process has occurred to avoid any compensatory physiological effects. In addition, such a strategy would be directly applicable to the clinical situation. Therefore, we will focus our discussion on the postdevelopmental genetic manipulations in hypertension.

**Knockdown Approach by Antisense**

First conceptual evidence that antisense (AS) could be effective in lowering high BP was derived with the use of AS oligonucleotides (AS-ODNs) targeting the RAS. AS-ODNs targeting angiotensinogen, angiotensin-converting enzyme (ACE), and angiotensin II (Ang II) type-1 receptor (AT1R) have been shown to lower high BP for several days in at least 3 different rat models of hypertension.5–9 Although impressive, this approach turns out to be little improvement over traditional pharmacotherapy. The next significant advancement was made with the use of viral vectors to deliver AS-ODN. Our research group was among the first to use retroviral vector-mediated systemic delivery of AT1R-AS in 5-day-old spontaneously hypertensive rats (SHR) produced lifelong antihypertensive effects without any visible side effects. High BP and cardiac and vascular pathophysiologies were prevented from developing throughout the life of the SHR. These observations were the first to provide “proof of principle” for hypertension gene therapy. This concept is not restricted to the SHR and has now been proven to be successful with the use of both genetic and nongenetic animal models of hypertension.10–12 These studies demonstrated that a single intracardiac injection of retroviral vector containing AT1R-AS in 5-day-old spontaneously hypertensive rats (SHR) produced lifelong antihypertensive effects without any visible side effects. High BP and cardiac and vascular pathophysiologies were prevented from developing throughout the life of the SHR. These observations were the first to provide “proof of principle” for hypertension gene therapy. This concept is not restricted to the SHR and has now been proven to be successful with the use of both genetic and nongenetic animal models of hypertension.10–12

In addition, the targeting of other genes linked to high BP and hypertension produce similar antihypertensive effects, most notably angiotensinogen,16,17 β-adrenergic receptor,18,19 and epidermal growth factor receptor.20,21 Taken together, these observations establish that AS targeting is effective in the prevention of hypertension.

**Overexpression Approach**

Chao’s group22,23 has led the way in using the sense approach by overexpressing vasodilators, such as kallikrein, adrenomedullin,24 ANP,25 and eNOS,26 in several different experimental models of hypertension. Their studies have demonstrated that delivery of each gene, either by naked DNA or by using viral delivery methods, results in an impressive lowering of high BP and attenuation of the pathophysiology. For example, a single injection of plasmid-containing human tissue kallikrein gene in hypertensive rats effectively reduced high BP and resulted in a morphological improvements in kidney and cardiac pathophysiology.22,27,28 This was associated with increases in circulating kallikrein levels.29 Similarly, eNOS or adrenomedullin gene overexpression reduced high BP and provided protection against hypertension, cardiac hypertrophy, and renal damage in both salt-sensitive and volume-dependent hypertension.24,26,30 Although the effects were not always very prolonged, there were reductions in end-organ damage with these therapies. Finally, beneficial outcomes in high BP have been demonstrated with superoxide dismutase and heme oxygenase gene transfers in the SHR.31

With the rationale that Ang II type-2 receptor (AT2R) play a counterregulatory role to the AT1R-mediated effects, our group recently used the AT2R gene to determine whether its overexpression would lead to beneficial/protective effects on the cardiovascular system. AT2R gene transfer was carried out with lentiviral vector-mediated AT2R cDNA. Twenty-one weeks after gene transfer, the lenti-AT2R–treated SHR exhibited decreased left ventricular wall thickness and reduced cardiac hypertrophy.32 The antihypertrophic and antiremodeling effects by lenti-AT2R were reproduced in the Ang II–infusion rat model of hypertension.33 The beneficial outcomes were observed despite the persistent elevated BP in these animals indicating that AT2R gene transfer can protect the heart from hypertension-induced damage independent of high BP regulation.33

In summary, it is clearly evident that the gene therapy approach for hypertension is experimentally sound, intellectually exciting, and technically feasible and holds promise for the long-term control and, possibly, the cure for hypertension. However, many important issues must first be resolved before this strategy is deemed ready for clinical trials. Some of these issues include the following: (1) the reversal of established hypertension in experimental models; (2) the development of a vector system that can regulate transgene expression to match for individual degrees of disease severity and that can switch off transgene expression in case of adverse effects; (3) the discovery and/or general consensus for an ideal gene target for hypertension; and (4) the extensive safety evaluation of viral gene delivery systems.

**Future Directions**

The most critical issue that is detrimental to a successful transition of the gene therapy strategy from preclinical to clinical levels is the selection of a gene that could be easily and efficiently targeted in humans. Preclinical animal studies with the use of AS have been extremely successful and have been critical in providing a conceptual support for this strategy. Although impressive, the AS approach has many limitations and, therefore, may not be ideal for use in humans. For example, whereas the systemic delivery of viral vectors containing proven and safe-sense sequences is feasible, the systemic delivery of AS in humans is not desirable, because it may nonspecifically target genes at unintended sites. Moreover, AS gene transfer has been less effective for the reversal of hypertension as a result of its significant lower efficacy in adult animals.34 Finally, current technology does not allow to carryout high-efficiency AS gene transfers for which the expression can be regulated on demand. In contrast, the overexpression strategy holds the potential in overcoming these obstacles. In fact, overexpression of genes,
such as eNOS, kallikrein, ANP, and so forth, has been found to be effective in preclinical studies; however, their targeting does not correct the underlying cause, that is, a hyperactive RAS. Thus, we must select another target gene, which is more specific for BP control, genetically linked to hypertension and its pathophysiology, and easy to use in human gene transfer settings. Our current results indicate that the angiotensin-converting enzyme 2 (ACE2) gene possesses many advantages over its predecessors.

ACE2, a newly discovered member of the RAS, possesses 42% sequence homology with ACE. It is an ectoenzyme of which the catalytic site faces the extracellular space and is, thus, capable of hydrolyzing extracellular peptides. In addition, like ACE, ACE2 appears to be susceptible to cleavage and secretion from the cell surface and has a topology of a type-I integral membrane protein. In spite of these similarities, ACE2 is distinct from ACE. It possesses distinct substrate specificity and is not inhibited by ACE inhibitors (ACEi). ACE2 catalyzes the formation of Ang (1-9) from Ang I and Ang (1-7) from Ang II, thus playing a central role in balancing the vasoconstrictor activity of Ang II with the vasodilatory effects of Ang (1-7). In addition to its role in the formation of Ang (1-7), ACE2 acts with high-catalytic efficiency on various other vasoactive peptides, such as apelins, kinin metabolites, neuropeptides, and opioid peptides, such as dynorphin A (1-13). These observations have led many to propose that an increase in ACE2 would be beneficial for the cardiovascular system, whereas its deficiency may lead to cardiovascular pathophysologies and hypertension.

The following evidence additionally support this contention: (1) ACE2 gene maps to a defined QTL associated with hypertension in rat models; (2) two SNPs in the ACE2 gene are shown to be associated with human coronary artery disease; (3) Ang (1-7), a major product of ACE2, acts as a vasodilator, ACEi, and a possible inhibitor of the AT1R; (4) both ACE2 and its major product, Ang (1-7), are demonstrated to oppose proliferative and profibrotic effects of Ang II; (5) disruption of the ACE2 gene in mice results in an elevation of Ang II, impaired cardiac contractility, and induction of hypoxia-responsive genes in cardiac tissue; and (6) transgenic mice overexpressing ACE2 exhibit lower BP, whereas the decrease in ACE2 has been demonstrated in several animal models of hypertension. A recent review from our group provides detailed conceptual support for the potential role of ACE2 as a novel therapeutic target for hypertension and CVDs. Our initial experiments corroborate this view and are summarized below.

We have cloned both membrane-bound (mACE2) and secreted (sACE2) forms of ACE2 in the lentiviral vector. Systemic administration of lenti-mACE2 primarily transduces cardiovascular-relevant tissues, whereas shACE2 is primarily sequestered into the plasma. For the first time, this provides us with an approach where both plasma and tissue levels of ACE2 can be increased. Thus, availability of sACE2 in the lentiviral vector offers us the opportunity to test the feasibility of intramuscular gene delivery. This would circumvent limitations of AS delivery issues in humans. It is pertinent to mention that the feasibility of intramuscular gene delivery to overexpress secreted peptides has been successful in many instances. Finally, our studies have indicated that overexpression of ACE2 by systemic delivery of lentivector mACE2 is highly effective in the prevention of cardiac pathophysiology, including myocardial fibrosis associated with Ang II infusion–induced hypertension. In addition, long-term overexpression of ACE2 in the SHR is highly effective in the attenuation of high BP (unpublished data).

The next critical issue is the development of a regulatable viral gene delivery system exhibiting cellular and tissue selectivity. Also, an ideal promoter driving transgene expression would be active for prolonged periods and could be switched on or off at will. This is not only critical for the control of the degree of transgene expression and, therefore, the therapeutic response, but it is also a necessary component for the ability to turn off the expression as a result of any unforeseen side effects. A number of regulatable gene expression systems (ie, Tet on/off, CYP1a, and progesterone regulatable system) are currently available; however, their efficacy in hypertension research remains to be evaluated. Of course, an ideal regulatable system for hypertension should be based on a “vigilant” concept in which it would respond to alterations in pathophysiological parameters, such as high BP, vascular dysfunctions, and hypoxia in a cell-specific manner. In view of the fact that rapid progress is being made in the development of such systems and in their evaluation for in vivo effectiveness, it would not be long before they are available for hypertension research.

References


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*Hypertension*. 2006;47:6-9; originally published online December 12, 2005; doi: 10.1161/01.HYP.0000196685.91424.01

*Hypertension* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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