Is Gene Therapy for Hypertension Possible?

M. Ian Phillips

Because hypertension is a chronic disease of multifactorial and multigenetic origin, it has not been considered appropriate for gene therapy. Gene therapy has tended to be limited to diseases resulting from a single gene mutation that can be replaced by gene insertion or to life-threatening diseases for which there are few alternative treatments, such as glioma and other cancers and rampant HIV infection. Hypertension, in contrast, is controllable by current drug therapies, and although it is a significant risk factor for stroke and heart attack, it lacks the sense of urgency associated with other diseases. The multifactorial nature of hypertension presents a Gordian knot of too many tangles to untie. Gene studies to sort out linkages and associations with hypertension are progressing, but slowly. There have been positive correlations to the angiotensinogen gene and recently to the human G protein B3 subunit gene. Lifton has intensively studied some rare forms of single gene mutations that result in hypertension. However, the causes of essential hypertension remain enigmatic.

Nevertheless, drug companies have for years made advances in the control of hypertension with single pharmaceutical targets. The most successful drugs developed to reduce hypertension have been designed to inhibit β-receptors, angiotensin-converting enzyme (ACE) receptors, and recently angiotensin type 1 receptors (AT₁-R). Thus, without a genetic linkage study and without a solution to the multifactorial nature of hypertension, drug studies have shown that inhibiting proteins that are overabundant or overactive in plasma or tissue reduces hypertension. The mechanisms of these drug actions are not thoroughly understood. Despite years of β-blocker use for the treatment of hypertension, there has been no generally agreed on mechanism to explain the antihypertensive effects of this class of drugs, yet the Joint National Committee on the treatment of hypertension has regularly recommended their use as a first-line pharmacological approach to controlling hypertension. The ACE inhibitors have been stellar performers in the treatment of hypertension, quite beyond the initial logic for their use. Although they are designed to inhibit the production of angiotensin II and therefore presumably reduce the vasoconstrictive effects of that octapeptide, ACE inhibitors are effective in both high-renin and low-renin forms of hypertension.

The latest entries into drug treatment for hypertension are the AT₁-R blockers or AT₁-R inhibitors. Although the debate continues regarding which type of drug is better, both ACE inhibitors and AT₁ antagonists are effective in controlling high blood pressure (BP).

Thus, for gene therapy, it is not necessary to analyze all the possible genes involved in hypertension because we can target those genes that have already been the targets of successful drug treatments. The next question that follows is: What is the need for gene therapy for hypertension? One answer is that drugs currently used have to be taken daily (and some even more frequently) to be effective. Therefore, there is a need for a therapeutic approach that would produce a long-lasting effect without patient compliance of taking pills daily. It is estimated that only 20% to 35% of all patients treated for hypertension have their BP under control. Second, the drugs have side effects that some patients cannot tolerate. Because drugs are artificial to human physiology, it is intuitively appealing to use a biological means, such as gene therapy, to treat a biological problem, particularly if that gene therapy could be made highly specific to a target tissue and its activity. I would suggest that most hypertensive patients would welcome a safe, long-term treatment that they needed to renew only once a year, or less frequently, to provided them with excellent control of their BP without side effects. The question becomes: Is that utopian desire possible? Success in recent experiments on laboratory animals suggests that gene modification can work in reversing hypertension.

Increase Vasodilation or Inhibit Vasoconstriction?

Strategies for hypertension gene therapy require either inserting extra gene copies of genes associated with ameliorating effects, such as vasodilation and reduced hypertrophy, or inhibiting genes associated with exacerbating effects, such as vasoconstriction and growth promotion (Figure). As our knowledge of gene products involved in controlling the cardiovascular system expands, gene therapy for cardiovascular diseases, and hypertension in particular, presents several alternative choices of candidate genes to target. Knockout of genes of the renin-angiotensin system (RAS) in mice reduced BP, and knockout of genes for atrial natriuretic peptide and the kallikrein system increased BP in mice. Inserting renin genes in rats and increasing renin substrate in mice increased BP. The “vasodilator” gene approach is being tested by introducing genes for expression of atrial natriuretic peptide, endothelial nitric oxide synthase, and human tissue kallikrein in plasmids. A DNA construct with human endothelial nitric oxide synthase gene driven by a cytomegalovirus (CMV) promoter injected in the tail vein.
of spontaneously hypertensive rats (SHR) reduced BP for 2 to 12 weeks. Similarly, a single injection of plasmid containing human tissue kallikrein gene in adult or newborn SHR effectively reduced BP for up to 10 weeks. In Dahl salt-sensitive rats, another model of hypertension, intravenous injection of adenovirus vector with kallikrein gene (a full-length cDNA) and under CMV promoter produced not only a reduction in BP but morphological improvements in kidney pathology and reduction of cardiac mass. However, there is little clinical experience of increasing kallikreins or atrial natriuretic peptide to treat hypertension, and there are many unknowns in this approach. Much more is known about inhibition of vasoconstriction clinically. Therefore, we have used antisense (AS) inhibition as an alternative to clinically useful RAS antagonists. Two strategies are being used. One is the direct administration of AS oligodeoxynucleotides (ODN), and the other is viral vector delivery of AS-oriented DNA.

**The Antisense Oligonucleotide Approach**

The first approach at modifying genes to lower hypertension was with AS ODN, directed to the AT1-R and angiotensin mRNA. AS ODN are single-stranded, short sequences of nucleotides encoding DNA that are complementary to specific mRNA. They inhibit specific protein synthesis by reducing translation or transcription. The promise of the AS approach is that its high specificity should result in fewer side effects than those produced by drugs. Because AS action is prolonged, dosing could be once a week.

Oligomers are designed to hybridize to the mRNA. The hybridized AS ODN complex prevents a specific mRNA from translating its message into a cellular protein product because it is unable to read through the ribosome. The presence of a deoxyribose nucleotide sequence generally (but not always) stimulates RNAse H to hydrolyze specific RNA covered by the AS ODN, which reduces the numbers of mRNA copies and frees the AS ODN to hybridize again. The design of AS ODN, however, is complicated by lack of theory concerning the annealing and folding of certain nucleotide sequences. To produce effective AS ODN for in vivo use, we construct three 15- or 18-nucleotide sequences complementary to encompassing the initiation codon (AUG) site or downstream sites of the mRNA. Although phosphodiester oligonucleotides are effective in vivo, they have a short duration. For longer-lasting action, oligomers were modified with a phosphorothioated backbone. Several published reviews have been critical of phosphorothioate modifications because of the risk of toxic effect of sulfur. This can be avoided by using short oligomers (<22-mer). Short phosphorothioated oligomers work well in vivo and are avidly taken up by cells. Shorter lengths (<9-mer) may lose specificity and be vulnerable to nucleases.

Effective AS ODN have been targeted to mRNA for renin, angiotensinogen, ACE, and AT1-R. They have been tested in 3 different models of hypertension, including the genetic model (SHR), a surgical model (2-kidney, 1 clip hypertension [2K1C]), and an environmental stress model (cold-induced hypertension) (Table 1). In every case in which we have tested the selected AS oligomers, there have been consistent biological signs of gene knockdown. High BP decreases 20 to 30 mm Hg within 3 to 9 hours after AS ODN administration. The effect is long lasting compared with any drug that is currently available. A single dose of AS reduces BP for up to 7 days, with a mean of 3 to 4 days. Similar

**TABLE 1. Antisense Oligonucleotides That Reduce Hypertension**

<table>
<thead>
<tr>
<th>Target mRNA</th>
<th>Model</th>
<th>Route</th>
<th>Effect*</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>AT1-R</td>
<td>SHR</td>
<td>ICV</td>
<td>−30 mm Hg</td>
<td>Gyurko et al, 1993</td>
</tr>
<tr>
<td>Angiotensinogen</td>
<td>SHR</td>
<td>ICV</td>
<td>−35 mm Hg</td>
<td>Phillips et al, 1994</td>
</tr>
<tr>
<td>C-fos</td>
<td>SHR</td>
<td>ICV</td>
<td>−16 mm Hg</td>
<td>Suzuki et al, 1994</td>
</tr>
<tr>
<td>Thyrotropin-releasing hormone</td>
<td>SHR</td>
<td>Intrathecal</td>
<td>−38 mm Hg</td>
<td>Suzuki et al, 1995</td>
</tr>
<tr>
<td>Angiotensinogen</td>
<td>SHR</td>
<td>ICV</td>
<td>−37 mm Hg</td>
<td>Wielbo et al, 1996</td>
</tr>
<tr>
<td></td>
<td>SHR</td>
<td>Hepatic vein</td>
<td>−25 mm Hg</td>
<td></td>
</tr>
<tr>
<td>Angiotensinogen</td>
<td>SHR</td>
<td>Hepatic vein</td>
<td>−20 mm Hg for 4 d</td>
<td>Tomita et al, 1995</td>
</tr>
<tr>
<td>AT1-R</td>
<td>SHR</td>
<td>ICV</td>
<td>−20 to −30 mm Hg for 5 d</td>
<td>Gyurko et al, 1997</td>
</tr>
<tr>
<td>Angiotensinogen</td>
<td>SHR</td>
<td>IV</td>
<td>−30 mm Hg for 5 d</td>
<td>Makino et al, 1986</td>
</tr>
<tr>
<td>AT1-R</td>
<td>2K1C</td>
<td>IV</td>
<td>−30 to −40 mm Hg for 5–7 d</td>
<td>Galli et al, 1998</td>
</tr>
<tr>
<td>AT1-R</td>
<td>CIH</td>
<td>ICV</td>
<td>−34 mm Hg for 3–5 d</td>
<td>Peng et al, 1998</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IV</td>
<td>−38 mm Hg</td>
<td></td>
</tr>
</tbody>
</table>

ICV indicates intracerebroventricular; IV, intravenous; and CIH, cold-induced hypertension.

*Change in systolic BP.
results have been reported for angiotensinogen AS ODN.\textsuperscript{24} Control ODN of either sense or scrambled sequences of the same length do not decrease BP. The hypertensive effect of AS treatment can be maintained with repeated administration. Beneficial effects on the heart and kidneys still need to be tested. The naked AS oligonucleotide is effective, but cat-ionic liposome carriers\textsuperscript{22,23} or asialoglycoprotein\textsuperscript{24} increases the effectiveness of the AS injected intravenously. The oligonucleotides we used do not appear to be toxic even after repeated injections over months. Pharmacokinetics show that the effectiveness of the AS injected intravenously. The AS approach would provide longer-lasting effects than current drugs, but oral delivery has yet to be developed. AS treatment can be maintained with repeated administration. AS ODN in normoten-
sive animals produced little or no reduction in BP. This is presumably because the AS treatment reduces overexpression of genes of the RAS that contribute to hypertension. Since the uptake of ODN in liver is high, angiotensinogen AS is ideally suited because angiotensinogen is primarily produced in the liver. Similarly, the kidneys and blood vessels, which are rich in AT\textsubscript{1}-R, are appropriate targets for the AT\textsubscript{1}-R AS ODN. ACE AS ODN might be delivered to the lungs, a major site of ACE action, via nasal or oral spray. The advent of AS therapy in general is here, with completion of phase 3 clinical trials with AS ODN showing low toxicity and a good therapeutic index,\textsuperscript{25} making these ideas acceptable clinically. The AS approach would provide longer-lasting effects than current drugs, but oral delivery has yet to be developed. AS does not cross the blood-brain barrier or the placenta, which may be advantageous for therapy without central side effects or in preeclampsia without compromising the fetus. For very prolonged effects, however, the delivery of AS will be in a viral vector (Table 2).

Viral Vectors for Antisense DNA Delivery

Several viruses have been tested for gene delivery, and each has its advantages but does not perfectly fit the description of the “ideal viral vector.”\textsuperscript{28,29} To be the perfect vector, a virus should fulfill all of the following criteria.

The vector should be safe. This means that it cannot be a virus known to cause disease, or it has to be re-engineered to be harmless. The viral vector should not elicit an immune or inflammatory response. It should not integrate into the genome randomly and risk disrupting other cellular genes and cause mutagenesis. The virus also has to be replication deficient for prevention of the spread to other tissues or the infection of other individuals. An ideal vector would deliver a defined gene copy number into each infected cell.

In addition, the vector must be efficiently taken up in target tissue. The virus has to infect the target cells with high frequency to achieve biological effect.

To be practical, the vector should be easy to manipulate and produce in pure form. The virus should be able to accommodate the transgene and a promoter. The recombinant vector should have a high titer. The recombinant vector DNA must be packaged with high efficiency into the viral capsid proteins.

Retroviral Vectors

Retroviruses have a single-stranded RNA genome that is converted to DNA in a cell by reverse transcriptase. The DNA is incorporated into the host genome and expresses RNA. Retroviruses have been used primarily because of their high efficiency in delivering genes to dividing cells. Retroviruses permit insertion and stable integration of single-copy genes. Although effective in cell culture systems, they randomly integrate into the genome, which raises concerns about their safety for practical use in vivo. Retroviruses can only act in dividing cells, which makes them ideal for tumor therapy but less desirable at sites where other cells are dividing that need to be protected. In hypertension research they are being investigated in developing SHR. Iyer et al\textsuperscript{30} reported that a retrovirus (LNSV) with AS AT\textsubscript{1}-R injected into newborn SHR prevents the development of hypertension in adults. Several weeks after the vector was injected, hypertension failed to develop. However, polymerase chain reaction analysis of the vector DNA showed that the virus had disappeared by 30 days, indicating that the long-term effect was due to exposure of a developing RAS to AS for the AT\textsubscript{1}-R during a critical period of development. Nevertheless, this had beneficial effects, and a repeat of the experiment showed that the AS AT\textsubscript{1}-R had prevented the development not only of hypertension but also of cardiac fibrosis.\textsuperscript{31} Retroviruses are appropriate for dividing cells but are limited by their lack of effect in nondividing cells and therefore cannot be used for hypertension therapy in adults, although they may be useful for cardiomyopathy, restenosis, and vascular remodeling.

Adenoviruses

Adenovirus vectors have been tested successfully in vascular gene transfer in isolation.\textsuperscript{32–35} Adenovirus is a double-stranded DNA with 2700 distinct adenoviral gene products. The virus infects most mammalian cell types because most cells have membrane receptors. They enter the cell by a receptor-induced endocytosis and translocate to the nucleus. Most adenovirus vectors in their current form are episomal,
ie, they do not integrate into the host DNA. They provide high levels of expression, but the episomal DNA invariably turns inactive. In some species, e.g., mice, this may be a long time compared with their life span, but in humans it is a limitation of the virus as a vector. Because the adenoviral genes express hundreds of proteins, adenovirus stimulates the immune system and vascular inflammation. This could be offset by coadministration of immunosuppression agents, but this would probably defeat the purpose of gene therapy by introducing side effects. Repeated infections result in inflammatory response with consequent tissue damage. This limitation makes current recombinant adenovirus unsuitable for long-term treatment in humans, and several gene therapy trials using adenovirus vectors have failed to produce acceptable results. The adenovirus is easy to produce and therefore useful for experimental animal studies of mechanisms. Adenovirus, as a vector for human kallikrein gene with a CMV promoter, has been shown to delay the development of 2K1C hypertension in rats and in young (8-week-old) SHR. A single intravenous injection of this vector reduced BP for >24 days in the 2K1C rats and for 40 days in SHR, compared with an adenovirus vector with a reporter gene only. There was a concomitant reduction in cardiac hypertrophy and improved renal blood flow. The hypothesis for the mechanism is that the kallikrein gene expresses endogenous kinins that counteract the vasoconstrictive and growth effects of increased angiotensin II induced by renal artery stenosis. However, the serum and urine kallikrein was no longer detectable at 30 days in the SHR.

The adenovirus as a vector has too many limitations at present to be successful in human gene therapy for chronic disease, although further engineering of the adenovirus may eventually avoid these limitations.

**Adeno-Associated Virus**

The adeno-associated virus (AAV) has been gaining attention because of its safety, lack of immunogenic viral proteins, and efficiency. It has been successfully used for delivering AS RNA against α-globin and HIV long terminal repeat and in phase 1 clinical trials for cystic fibrosis. It is our vector of choice for delivering AS targeted to the AT1-R in hypertensive rat models. AAV is a parvovirus, discovered as contamination of adeno viral stocks. It is a ubiquitous virus that has not been linked to any disease. It is also classified as a dependovirus, because its replication is dependent on the presence of a helper virus.

On infection of a human cell, the wild-type AAV integrates to the q arm of chromosome 19. Although chromosomal integration requires the terminal repeats, the viral components responsible for site-specific integration have been recently targeted to the rep proteins. With no helper virus present, AAV infection remains latent indefinitely. To produce recombinant AAV (rAAV), helper viruses (adenovirus and plasmids with rep and cap genes) are required.

Several factors prompted researchers to study the possibility of using rAAV as an expression vector. One is that the requirement for delivering a gene is surprisingly little. All that is necessary are the 145-bp inverted terminal repeats, which are only 6% of the AAV genome. This leaves room in the vector to assemble up to 4.4 kb DNA insertion. While this carrying capacity may limit the AAV to delivering large genes, it is amply suited to delivering small genes and particularly AS cDNA, which can be shorter than the full-length DNA required for a gene. The AAV has sufficient loading capability to carry the AS DNA, plus a potent promoter to drive the DNA and a reporter gene, such as a neomycin resistance gene, fused with its appropriate tyrosine kinase promoter.

The second characteristic that makes AAV a good vector candidate is its safety. There is a relatively complicated rescue mechanism, as described above. Not only adenovirus (wild type) but also AAV genes are required to mobilize the rAAV. This limits the spread of rAAV vectors from target areas to nontarget areas in tissues. AAV is not pathogenic and not associated with disease. The removal of viral coding sequences in producing rAAV removes the source of immune reactions to viral gene expression, and therefore rAAV does not evoke an inflammatory response (in contrast to the recombinant adenovirus).

AAV is also a good candidate for gene therapy because it has a very broad host range. AAV infects all mammalian tissues tested. The AAV remains intact for long periods of time. We have noted rAAV-AS expression in vascular smooth muscle for >9 weeks and in brain for >11 months. This may be related to the genomic integration of rAAV.

The limitation of AAV is its production. Although it can be purified and concentrated, which are advantages, it also has to be rendered free of adenovirus, and therefore production is more complicated than for other vectors. High titers and large quantities are needed for intravenous injections.

The advantages, particularly its safety, make AAV appear to be one of the best candidates currently for delivery of genes for long-term therapy, especially because it has been shown to be nontoxic in cystic fibrosis patients.

**Recombinant Adeno-Associated Virus With Antisense**

To prolong AS action beyond the 3 to 7 days of effectiveness with ODN and to avoid all concerns about the toxicity of liposome carriers and accumulation of sulfur from the phosphorothioated backbones of the oligonucleotides, we developed a viral vector delivery of cDNA in the AS direction. A rAAV containing cDNA for AT1-R in the AS direction with a CMV promoter and a neomycin resistance gene (neo) as a reporter gene was constructed and shown to reduce AT1-R in vitro. The rAAV was injected in the brain or intravascularly in SHR. High BP was reduced in adult SHR with a single injection of this rAAV. No effect on heart rate was seen, and no toxic effects were noted in the animals. The decrease in high BP in adult SHR lasted for at least 2 months. By comparison, there was no effect on BP of rAAV without the AS DNA and only a CMV promoter and a reporter gene (green fluorescent protein). Systemic application of the rAAV-AS vector in young SHR (2 to 5 weeks) injected once, intracardially, slowed the development of hypertension and reduced BP compared with controls for over 2 months. Since a single application of rAAV expresses...
transgenes for 6 months to 1 year in animals. This would translate into even longer time effects in humans. The reduction in BP with AAV-AS-AT\(_1\)-R began 1 to 2 weeks after the injection of the viral vector. At 2 weeks after vector administration, reverse transcription–polymerase chain reaction analysis of RNA extracted from liver, heart, kidney, and aorta showed expression of AS mRNA. An unexpected plus to the gene therapy in this model was the reduction in left ventricular hypertrophy often associated with hypertension and heart failure. The integration of rAAV provides genomic AS AT\(_1\)-R mRNA so that there is long-term stability for transgene production.

The mechanism of AS gene action is the expression of cellular AS AT\(_1\)-R mRNA by the transgene. The AS RNA hybridizes with the endogenous AT\(_1\) receptor mRNA and inhibits production of the AT\(_1\)-R protein. Since this action is competitive, the reduction in protein is never complete. However, since the goal of this approach is to reduce but not eliminate AT\(_1\)-R, so that normal physiology of the RAS is intact, the rAAV-AS-AT\(_1\)-R approach is a reasonable one. No mutations have been observed. The continuous production of AS AT\(_1\)-R over weeks and months offers a potential antihypertension therapy without the peaks and valleys of BP fluctuations associated with daily drug regimens.

Promoters

In addition to the choice of vectors, there is also a choice of promoters. The ideal promoter would be active for prolonged periods to maintain transgene expression and would be specific for a given tissue cell type, such as heart, kidney, or vascular smooth muscle. Tissue promoters for rAAV angiotensinogen in liver and specific promoters for rAAV AT\(_1\)-R in vascular smooth muscle cell are being studied. For human gene therapy, it will be desirable to target the vector to specific tissues and develop the possibility of switching the genes on or off by controlling their promoters. There are few data on this possibility that can be discussed, at present, without being speculative. For hypertension there will be occasions when switching off the gene therapy may be important. Ultimately, properly engineered gene therapy should have that option.

Conclusions

Two approaches to gene therapy for hypertension are currently in the experimental stage. One is the administration of AS oligonucleotides directed to gene targets that have been shown to be relevant by current drug therapy. The second is the use of viral vectors to deliver either more copies of “vasodilator genes” or to deliver AS DNA, directed to inhibiting “vasoconstrictor genes” (Figure). Clearly, there are many technical problems with respect to efficiency of transfer, choice of administration, and determination of uptake, lack of toxicity, lack of immune response, and clinical effectiveness. All these problems must be overcome before gene therapy for hypertension (or any disease) is acceptable. Equally clear is that the gene therapy approach is attractive as long as we have diseases that cannot be cured. The advantage of the AS ODN approach is that the oligonucleotides can be used and understood as pharmaceutical agents. They act within a few hours, can be measured systemically, and have a dose-response relationship. Vector delivery, by contrast, is far from being defined in pharmaceutical terms. Few studies on dose response and tissue distribution have been completed. The vasodilator gene strategy has been shown to reduce BP with a variety of genes, including human kallikrein, endothelial nitric oxide synthase, adenomedullin, and atrial natriuretic peptide in several different models of hypertension, including SHR, Dahl salt-sensitive, and 2K1C renovascular hypertensive models. The vasoconstrictor gene inhibition strategy with AS in DNA in rAAV appears to be effective for reducing high BP in animal models of hypertension, including SHR, for at least 2 months. Retrovirus delivery of AS AT\(_1\)-R prevents hypertension from developing in young SHR but may be limited to a developmental model because the vector infects only dividing cells. Eventually the development of these strategies could provide new antihypertensive options. Gene vectors would be superior to current drugs when a single administration of a harmless recombinant virus produces prolonged effects several months with minimal side effects, limited only to the direct effects of lowering the activity of the overexpressed gene.

Gene therapy for hypertension would give the patient continuous biological control over high BP. While a great deal more work needs to be done on the basic science of these approaches to gene therapy for hypertension before they can be applied to humans, the results are encouraging, the biology is feasible, and the medical possibility is plausible.

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References


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